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(54) Title: METHODS UTILIZING NOVEL TARGET GENES RELATED TO IMMUNE-MEDIATED DISEASES

(57) Abstract: The present invention provides methods utilizing novel target genes related to immune-mediated diseases, such as asthma, allergy and autoimmune diseases. The invention is based on a molecular level description of the polarization of CD4+ precursor cells (Thp) from which T helper cells are known to originate. Particularly, the present invention provides a method of identifying a compound capable of modulating the polarization of CD4+ lymphocytes. The invention is also related to a method for assessing the presence of, or a predisposition to, an immune-related disorder in a subject.

METHODS UTILIZING NOVEL TARGET GENES RELATED TO IMMUNE-MEDIATED DISEASES

FIELD OF THE INVENTION

The present invention provides methods utilizing novel target genes related to immune-mediated diseases, such as asthma, allergy and autoimmune diseases. The invention is based on a molecular level description of the polarization of CD4+ precursor cells (Thp) from which T helper cells are known to originate. T helper cell subtypes (Th1 and Th2) have an important role in the immune system. However, many pathological processes, such as allergies, are associated with the presence of T helper cells at the site of inflammation.

BACKGROUND

T helper cell subtypes Th1 and Th2 cells arise from a common precursor cell in response to triggering through the T cell receptor and cytokine receptors for IL-12 or IL-4. This leads to activation of complex signaling pathways. Disturbances in the balance between type 1 and type 2 responses can lead to certain immune-mediated diseases such as asthma, allergy and certain autoimmune diseases (1-3). Thus, it is important to understand how Th1 and Th2 cells are generated.

Th1 and Th2 cells are known to originate from naïve CD4+ precursor cells (Thp) after antigenic activation through the T cell receptor (TCR) and co-stimulatory molecules in a suitable cytokine milieu. The main cytokines orchestrating Th1 and Th2 development are IL-12 and IL-4, respectively. Triggering of the TCR and cytokine signaling leads to activation of complex, and to a large extent poorly understood, downstream signaling networks that finally result in maturation of the effector Th1 and Th2 cells (4, 5). IL-12 (and in human also IFNα) induces the Th1 type response by activating the Signal Transducer and Activator of Transcription 4 (STAT4) mediated signaling pathway (6-8). Some other cytokines such as IFNγ and IL-18 can also promote Th1 responses, especially in combination with IL-12. Th2 differentiation is induced by IL-4 through the STAT6 signaling pathway (9-12). GATA binding protein 3 (GATA-3), avian musculoaponeurotic fibrosarcoma (v-maf) AS42, oncogene homolog (c-maf) and T-box expressed in T cells (T-bet) are also among the most important factors regulating the early polarization of Th2 and Th1 cells respectively (11-16).

Another important cytokine involved in Th1 and Th2 differentiation is TGFβ. This immunosuppressive cytokine exhibits pleiotrophic activities in various cellular processes and, importantly, can suppress the differentiation of CD4+ cells into the Th1 and Th2 subtypes (17). However, similar to IL-12 and IL-4, the target genes and details of TGFβ downstream signaling are not clear.

To provide a basis for understanding the mechanism of action and molecular networks involved in the signaling of these cytokines, the early phase leading to polarization of Th1 and Th2 cells in the presence and absence of TGFβ was examined. As a result, genes differentially regulated in the cells induced to polarize to Th1 and Th2 subtypes in human were identified. Importantly, to our knowledge 77 of these genes have not been previously described to be involved in Th1 and Th2 cell differentiation. In addition, we have further clarified which of the genes involved in the early polarization of human Th1 and Th2 cells are targets of IL-12 and IL-4 regulation and which of them are also targets of immunosuppressive TGFβ. Wild type mouse cells or STAT6-knockout cells were used to further clarify the mechanism how IL-4 regulates gene expression through STAT6 signaling. These genes newly identified genes involved in Th1 and Th2 differentiation serve as therapeutic targets in achieving an appropriate balance between Th1 and Th2 responses.

SUMMARY

Certain methods of the invention are related to a method of identifying a compound capable of modulating the polarization of CD4+ lymphocytes. The method includes the steps of contacting the compound with naïve CD4+ lymphocytes, and then inducing the polarization of the cells. Further, a gene expression profile from the lymphocytes is prepared during the polarization, and the profile is compared to a baseline gene expression profile of CD4+ lymphocyte polarization as established in Table 1. A difference in the expression profiles of the target genes identifies a potential drug compound for the treatment of asthma or other immune-mediated diseases.

The invention is also related to a methods of identifying a compound that modulates the expression or activity of at least one target gene listed in Table 2 or Table 6. The methods include the steps of (a) incubating a cell that can express a protein from said gene or a cell that has said activity with a compound under conditions and for a time sufficient for the cell to express the protein or activity of said gene, when the compound is not present, (b) incubating a control cell under the same conditions and for the same time

without the compound, (c) measuring expression or activity of said gene in the cell in the presence of the compound; (d) measuring expression or activity of said gene in the control cell; and (e) comparing the amount of expression or activity of said gene in the presence and absence of the compound, wherein a difference in the level of expression or activity indicates that the compound modulates the expression of said gene.

Other methods that are provided are to identify a compound that modulates differentiation of a lymphocyte. These methods generally involve contacting a test cell capable of expressing one or more gene markers listed in Table 2 or Table 6 with a test compound. The expression level of the one or more gene markers in the test cell is determined. The expression level of these gene markers are than compared with the expression levels for these same markers in a control cell. In these methods, the test cell and the control cell are lymphocytes and the cellular state of the control cell is known. A difference in the expression level between the test and control cell is an indicator that the test compound is a modulator of lymphocyte differentiation.

Another embodiment of the invention relates to a method of treating a patient with asthma or other immune-mediated disease. The method of treatment comprises administering to the patient a pharmaceutical composition that alters the expression or activity of at least one gene listed in Table 2 or Table 6. In a preferred embodiment of the invention, the active compound of said pharmaceutical composition is identified by a method of the invention.

Methods for classifying a lymphocyte or assessing the cellular state of a lymphocytic cell are also provided. Certain of these methods involve providing a test sample derived from the lymphocyte, wherein the lymphocyte is capable of expressing one or more nucleic acid markers from the group consisting of those listed in one or more of the tables (*e.g.* Table 1 or Table 2 and/or Table 6). The expression level of the one or more markers in the test sample are determined and compared with the expression level of the same markers in a control sample. The control sample is derived from a lymphocytic cell whose cellular status is known. The lymphocyte is then classified on the basis of this comparison. In some instances, the methods involve classifying the lymphocyte as being a Th1 or Th2 type cell.

A variety of methods for diagnosing the presence of , or a predisposition to, an immune-related disease are provided as well. These methods generally involve determining the expression level of one or more nucleic acid markers in a test sample obtained from a subject. These markers are selected from the group consisting of those

listed in one or more of the tables (*e.g.* Table 1 or Table 2 and/or Table 6). The expression level of the one or more nucleic acid markers in the test sample is compared with the expression level of the same markers in a control sample whose immune status is known. The presence or absence of the immune disorder in the subject, or a predisposition to the immune disorder, is then diagnosed on the basis of this comparison.

A BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Expression of Th1 and Th2 marker genes. CD4+ cells were purified from human cord blood and were activated with plate-bound αCD3 (1000 ng/μl for goating) and 500 ng/μl soluble αCD28. The cells were further polarized with either 2.5 ng/ml of IL-12 for Th1 conditions or 10 ng/ml of IL-4 for Th2 conditions in the presence and absence of 3 ng/ml TGFβ. Part of the activated cells were cultured in "neutral conditions" without any polarizing cytokines. The cells were collected at the time points of 0 h and 48 h. RNA was isolated from the samples and cDNA was prepared. Expression of known marker genes IFNγ, T-bet and GATA-3 was measured from the samples using Real-Time RT-PCR to ensure the polarization of the cells to the Th1 and Th2 direction. The figure shows representative data from one of two repeated experiments.

FIGS. 2A-2F. The target genes of activation, IL-12, IL-4 and TGFβ. The CD4+ cells were purified from human cord blood and were activated with plate-bound αCD3 (1000 ng/μl for goating) and 500 ng/μl soluble αCD28. The cells were further polarized with either 2.5 ng/ml of IL-12 for Th1 conditions or 10 ng/ml of IL-4 for Th2 conditions in the presence and absence of 3 ng/ml TGFβ. Part of the activated cells were cultured in "neutral conditions" without any polarizing cytokines. The samples were collected at the time points of 0 h and 48 h. The cRNAs were prepared for oligonucleotide microarray hybridizations and the data was analyzed with the MAS5 program. To identify the target genes of different treatments, the expression profiles of the samples were compared to each other: FIG. 2A shows target genes of activation; FIG. 2B shows target genes of IL-12; FIG. 2C shows target genes of IL-4; FIG. 2D shows target genes of TGFβ in Th1 conditions; FIG. 2E shows target genes of TGFβ in Th2 conditions; and FIG. 2F shows genes differentially expressed by Th1- and Th2-induced cells. The tonal intensities in the figures indicate the differences (signal log ratio) between two treatments. All the irreproducible results or changes below 2-fold (signal log ratio <1) were excluded from the results. Higher cut off (signal log ratio ≥4) for the target genes of activation was used to

reduce the number of the genes. The functional groups of the genes are represented as numbers (1. Cell surface molecules, 2. Cytokines, chemokines and other ligands, 3. Enzymes and pathway molecules, 4. Structural molecules and intracellular trafficking, 5. Transcriptional regulation, 6. Unclassified).

FIG. 3. Validation of oligonucleotide microarray results with Real-Time RT-PCR. For validation of the oligonucleotide array results, long term Th1 and Th2 primary cultures were generated from four individuals as previously described. The priming was performed in the presence of 100 ng/ml PHA (Murex Diagnostics, Chatillon, France) and irradiated CD32-B7 transfected fibroblasts. Th1 cultures were supplemented with 2.5 ng/ml of IL-12 (R&D Systems, Minneapolis, MN). Th2 cultures were supplemented with 10 μg/ml of anti-IL-12 (R&D Systems) and 10 ng/ml of IL-4 (R&D System). After 48 hours of priming, 40 U/ml of IL-2 (R&D Systems) was added into the cultures to enhance the proliferation of the lymphocytes. Part of the cells were cultured without any polarizing cytokines in the presence of IL-2 alone. During polarization, samples were collected at time points 0 h, 6 h, 24 h, 48 h or 7 d. Real-time quantitative RT-PCR was performed to quantitate the gene expression levels of SATB1, TIP3, DUSP6, E4BP4 and GADD45β.

DETAILED DESCRIPTION

I. Definitions

The terms "nucleic acid," "polynucleotide" and "oligonucleotide" are used interchangeably and refer to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogues of natural nucleotides that hybridize to nucleic acids in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence includes the complementary sequence thereof.

The term "target nucleic acid" refers to a nucleic acid (often derived from a biological sample), to which a polynucleotide probe is designed to specifically hybridize. It is either the presence or absence of the target nucleic acid that is to be detected, or the amount of the target nucleic acid that is to be quantified. The target nucleic acid has a sequence that is complementary to the nucleic acid sequence of the corresponding probe directed to the target. The term target nucleic acid can refer to the specific subsequence of a larger nucleic acid to which the probe is directed or to the overall sequence (*e.g.*, gene or mRNA) whose expression level it is desired to detect.

A "probe" or "polynucleotide probe" is an nucleic acid capable of binding to a target nucleic acid of complementary sequence through one or more types of chemical bonds, usually through complementary base pairing, usually through hydrogen bond formation, thus forming a duplex structure. The probe binds or hybridizes to a "probe binding site." A probe can include natural (*i.e.*, A, G, C, or T) or modified bases (7-deazaguanosine, inosine, etc.). A probe can be an oligonucleotide which is a single-stranded DNA. Polynucleotide probes can be synthesized or produced from naturally occurring polynucleotides. In addition, the bases in a probe can be joined by a linkage other than a phosphodiester bond, so long as it does not interfere with hybridization. Thus, probes can include, for example, peptide nucleic acids in which the constituent bases are joined by peptide bonds rather than phosphodiester linkages (see, *e.g.*, Nielsen *et al.*, *Science* 254, 1497-1500 (1991)). Some probes can have leading and/or trailing sequences of noncomplementarity flanking a region of complementarity.

A "perfectly matched probe" has a sequence perfectly complementary to a particular target sequence. The probe is typically perfectly complementary to a portion (subsequence) of a target sequence. The term "mismatch probe" refer to probes whose sequence is deliberately selected not to be perfectly complementary to a particular target sequence.

A "primer" is a single-stranded oligonucleotide capable of acting as a point of initiation of template-directed DNA synthesis under appropriate conditions (*i.e.*, in the presence of four different nucleoside triphosphates and an agent for polymerization, such as, DNA or RNA polymerase or reverse transcriptase) in an appropriate buffer and at a suitable temperature. The appropriate length of a primer depends on the intended use of the primer but typically ranges from 15 to 30 nucleotides, although shorter or longer primers can be used as well. Short primer molecules generally require cooler temperatures to form sufficiently stable hybrid complexes with the template. A primer need not reflect the exact sequence of the template but must be sufficiently complementary to hybridize with a template. The term "primer site" refers to the area of the target DNA to which a primer hybridizes. The term "primer pair" means a set of primers including a 5' "upstream primer" that hybridizes with the 5' end of the DNA sequence to be amplified and a 3' "downstream primer" that hybridizes with the complement of the 3' end of the sequence to be amplified.

The term "complementary" means that one nucleic acid is identical to, or hybridizes selectively to, another nucleic acid molecule. Selectivity of hybridization exists

when hybridization occurs that is more selective than total lack of specificity. Typically, selective hybridization will occur when there is at least about 55% identity over a stretch of at least 14-25 nucleotides, preferably at least 65%, more preferably at least 75%, and most preferably at least 90%. Preferably, one nucleic acid hybridizes specifically to the other nucleic acid. See M. Kanehisa, *Nucleic Acids Res.* 12:203 (1984).

The terms "polypeptide," "peptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues. The term also applies to amino acid polymers in which one or more amino acids are chemical analogues of a corresponding naturally occurring amino acids.

A "subsequence" or "segment" refers to a sequence of nucleotides or amino acids that comprise a part of a longer sequence of nucleotides or amino acids (*e.g.*, a polypeptide), respectively.

The term "operably linked" refers to functional linkage between a nucleic acid expression control sequence (such as a promoter, signal sequence, or array of transcription factor binding sites) and a second polynucleotide, wherein the expression control sequence affects transcription and/or translation of the second polynucleotide.

The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptides, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm such as those described below for example, or by visual inspection.

The phrase "substantially identical," in the context of two nucleic acids or polypeptides, refers to two or more sequences or subsequences that have at least 75%, preferably at least 85%, more preferably at least 90%, 95% or higher nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm such as those described below for example, or by visual inspection. Preferably, the substantial identity exists over a region of the sequences that is at least about 30 residues in length, preferably over a longer region than 50 residues, more preferably at least about 70 residues, and most preferably the sequences are substantially identical over the full length of the sequences being compared, such as the coding region of a nucleotide for example. For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm

program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (*see, e.g., Current Protocols in Molecular Biology* (Ausubel *et al.,* 1995 supplement).

One useful algorithm for conducting sequence comparisons is PILEUP. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, *J. Mol. Evol.* 35:351-360 (1987). The method used is similar to the method described by Higgins & Sharp, *CABIOS* 5:151-153 (1989). Using PILEUP, a reference sequence is compared to other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps. PILEUP can be obtained from the GCG sequence analysis software package, *e.g.*, version 7.0 (Devereaux *et al., Nuc. Acids Res*. 12:387-395 (1984).

Another example of algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST and the BLAST 2.0 algorithms, which are described in Altschul *et al., J. Mol. Biol.* 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al, supra.*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the

word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached.

For identifying whether a nucleic acid or polypeptide is within the scope of the invention, the default parameters of the BLAST programs are suitable. The BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, an expectation (E) of 10, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word length (W) of 3, an expectation (E) of 10, and the BLOSUM 62 scoring matrix. The TBLATN program (using protein sequence for nucleotide sequence) uses as defaults a word length (W) of 3, an expectation (E) of 10, and a BLOSUM 62 scoring matrix. (*See, e.g.,* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)).

Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions. "Bind(s) substantially" refers to complementary hybridization between a probe nucleic acid and a target nucleic acid and embraces minor mismatches that can be accommodated by reducing the stringency of the hybridization media to achieve the desired detection of the target polynucleotide sequence. The phrase "hybridizing specifically to", refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex mixture (*e.g.,* total cellular) DNA or RNA.

The term "stringent conditions" refers to conditions under which a probe will hybridize to its target subsequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. Generally, stringent conditions are selected to be about 5 °C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. (As the target sequences are generally present in excess, at Tm, 50% of the probes are occupied at equilibrium). Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30 °C for short probes (*e.g.,* 10 to 50

nucleotides) and at least about 60 °C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions can also be achieved with the addition of destabilizing agents such as formamide.

A further indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid, as described below. The phrases "specifically binds to a protein" or "specifically immunoreactive with," when referring to an antibody refers to a binding reaction which is determinative of the presence of the protein in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, a specified antibody binds preferentially to a particular protein and does not bind in a significant amount to other proteins present in the sample. Specific binding to a protein under such conditions requires an antibody that is selected for its specificity for a particular protein. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. *See, e.g.*, Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

"Conservatively modified variations" of a particular polynucleotide sequence refers to those polynucleotides that encode identical or essentially identical amino acid sequences, or where the polynucleotide does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given polypeptide. For instance, the codons CGU, CGC, CGA, CGG, AGA, and AGG all encode the amino acid arginine. Thus, at every position where an arginine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of "conservatively modified variations." Every polynucleotide sequence described herein which encodes a polypeptide also describes every possible silent variation, except where otherwise noted. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule by standard techniques. Accordingly, each "silent

variation" of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

A polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. A "conservative substitution," when describing a protein, refers to a change in the amino acid composition of the protein that does not substantially alter the protein's activity. Thus, "conservatively modified variations" of a particular amino acid sequence refers to amino acid substitutions of those amino acids that are not critical for protein activity or substitution of amino acids with other amino acids having similar properties (*e.g.,* acidic, basic, positively or negatively charged, polar or non-polar, *etc.*) such that the substitutions of even critical amino acids do not substantially alter activity. Conservative substitution tables providing functionally similar amino acids are well-known in the art. *See, e.g.,* Creighton (1984) *Proteins*, W.H. Freeman and Company. In addition, individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids in an encoded sequence are also "conservatively modified variations."

The term "naturally occurring" as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism that can be isolated from a source in nature and which has not been intentionally modified by humans in the laboratory is naturally occurring.

The term "antibody" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes or fragments of immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

A typical immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (VL) and variable heavy chain (VH) refer to these light and heavy chains respectively.

Antibodies exist as intact immunoglobulins or as a number of well- characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab)'_2$, a dimer of Fab which itself is a light chain joined to VH-CH1 by a disulfide bond. The $F(ab)'_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region thereby converting the $(Fab')_2$ dimer into an Fab' monomer. The Fab' monomer is essentially an Fab with part of the hinge region (*see, Fundamental Immunology*, W.E. Paul, ed., Raven Press, N.Y. (1993), for a more detailed description of other antibody fragments). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such Fab' fragments may be synthesized de novo either chemically or by utilizing recombinant DNA methodology. Thus, the term antibody, as used herein also includes antibody fragments either produced by the modification of whole antibodies or synthesized de novo using recombinant DNA methodologies. Preferred antibodies include single chain antibodies, more preferably single chain Fv (scFv) antibodies in which a variable heavy and a variable light chain are joined together (directly or through a peptide linker) to form a continuous polypeptide.

A single chain Fv ("scFv" or "scFv") polypeptide is a covalently linked VH::VL heterodimer which may be expressed from a nucleic acid including VH- and VL- encoding sequences either joined directly or joined by a peptide-encoding linker. Huston, et al. *Proc. Nat. Acad. Sci. USA*, 85:5879-5883 (1988). A number of structures for converting the naturally aggregated-- but chemically separated light and heavy polypeptide chains from an antibody V region into an scFv molecule which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. *See, e.g.* U.S. Patent Nos. 5,091,513 and 5,132,405 and 4,956,778.

An "antigen-binding site" or "binding portion" refers to the part of an immunoglobulin molecule that participates in antigen binding. The antigen binding site is formed by amino acid residues of the N-terminal variable ("V") regions of the heavy ("H") and light ("L") chains. Three highly divergent stretches within the V regions of the heavy and light chains are referred to as "hypervariable regions" which are interposed between more conserved flanking stretches known as "framework regions" or "FRs". Thus, the term "FR" refers to amino acid sequences that are naturally found between and adjacent to hypervariable regions in immunoglobulins. In an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen binding

"surface". This surface mediates recognition and binding of the target antigen. The three hypervariable regions of each of the heavy and light chains are referred to as "complementarity determining regions" or "CDRs" and are characterized, for example by Kabat et al. *Sequences of proteins of immunological interest*, 4th ed. U.S. Dept. Health and Human Services, Public Health Services, Bethesda, MD (1987).

The term "antigenic determinant" refers to the particular chemical group of a molecule that confers antigenic specificity.

The term "epitope" generally refers to that portion of an antigen that interacts with an antibody. More specifically, the term epitope includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Specific binding exists when the dissociation constant for antibody binding to an antigen is $\leq 1\mu M$, preferably ≤ 100 nM and most preferably ≤ 1 nM. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids and typically have specific three dimensional structural characteristics, as well as specific charge characteristics.

The term "specific binding" (and equivalent phrases) refers to the ability of a binding moiety (*e.g.*, a receptor, antibody, ligand or antiligand) to bind preferentially to a particular target molecule (*e.g.*, ligand or antigen) in the presence of a heterogeneous population of proteins and other biologics (*i.e.,* without significant binding to other components present in a test sample). Typically, specific binding between two entities, such as a ligand and a receptor, means a binding affinity of at least about 10^6 M^{-1}, and preferably at least about 10^7, 10^8, 10^9, or 10^{10} M^{-1}.

A "subject" generally refers to an organism from which lymphocytes can be obtained. Usually the subject is a mammal. The mammal can be a primate (e.g., a human, monkey, ape, or chimpanzee), or a non-primate (e.g., a mouse).

II. Overview

Many biological functions are controlled through changes in the expression of various genes by transcriptional (e.g., through control of initiation, RNA processing, etc.) and/or translational control. For example, fundamental biological processes such as cell cycle, cell differentiation and cell death, are often characterized by the variations in the expression levels of groups of genes (see e.g. WO02059271). The changes in gene expression also are associated with pathogenesis. Thus, changes in the expression levels of particular genes can indicate the presence and progression of various diseases.

According to the invention, genes that are differentially expressed during cytokine induced CD4+ lymphocyte polarization in both the presence and absence of TGFß have been discovered. One or more of these target genes can be used as part of an "an expression profile" that is representative of a particular state of a lymphocyte. Identification of these new target genes enable immune-mediated diseases to be analyzed more reliably. These results also provide new insights into T cell differentiation and reveal new potential target genes for the therapy of diseases such as asthma. These differentially expressed genes and their corresponding proteins can also be utilized as "markers" that characterize particular cellular states for lymphocytes.

The differentially expressed genes that have been identified can be utilized in a variety of methods for classifying lymphocytes, as well as diagnosing and treating immune-mediated diseases (e.g., asthma, allergic responses and autoimmune diseases). Kits and devices including one or more of the differentially expressed genes, proteins encoded by these genes and/or antibodies that bind the proteins are also provided.

For example, the differentially expressed genes can be used to in screening methods to identify compounds that modulate the expression or activity of the differentially expressed genes. Such methods can be utilized, for example, for the identification of compounds that can treat symptoms of disorders related to expression of proteins encoded by the differentially expressed genes. In addition, the invention encompasses methods for treating immune-mediated diseases or disorders by administering compounds and/or other substances that modulate the activity of one or more of the target genes or target gene products. Such compounds and other substances can effect the modulation either on the level of target gene expression or target protein activity. Certain classification methods that are also provided involve determining the level of one or more of the differentially expressed genes to determine whether a lymphocyte has been polarized in the Th1 or Th2 direction.

III. Differentially Expressed Genes

As described more fully in the examples below, an initial set of experiments were conducted to identify the gene expression profiles of CD4+ cells induced in the Th1 and Th2 directions in the presence and absence of TGFß. This allowed those genes involved in early polarization to be identified, as well providing insight regarding which genes are involved in the immunosuppressive effect of TGFß. Another set of experiments was then conducted to identify those genes in lymphocytes that are regulated by the cytokines IL-12

and IL-4. Most of the differentially expressed genes were identified using oligonucleotide arrays; the differential expression of certain genes was confirmed using real-time PCR approaches.

The differentially expressed genes include, for instance, those identified under the following sets of conditions or states:

(a) CD4+ cells activated by contact with CD3/CD28 versus unactivated CD4+ cells; these genes correspond to target genes of activation (*see, e.g.* FIG. 2A);

(b) activated CD4+ cells further activated with IL-12 versus activated CD4+ cells; these genes are representative of Th1 cells and correspond to target genes of IL-12 (*see, e.g.* FIG. 2B);

c) activated CD4+ cells further activated with IL-4 versus activated CD4+ cells; these genes are representative of Th2 cells and correspond to target genes of IL-4 (*see, e.g.* FIG. 2C);

(d) activated cells polarized with IL-12 exposed to TGFß versus similar cells not exposed to TGFß; these correspond to genes in Th1 cells that are affected by TGFß (*see, e.g.* FIG. 2D);

(e) activated cells polarized with IL-4 exposed to TGFß versus similar cells not exposed to TGFß; these correspond to genes in Th2 cells that are affected by TGFß (*see, e.g.* FIG. 2E); and

(f) genes differentially expressed in Th1 cells versus Th2 cells (*see, e.g.* FIG. 2F).

As discussed in greater detail below, knowledge of the nucleic acids that are up-regulated or down-regulated in the various types of lymphocytes and in different cellular states provides the basis for a number of different screening, treatment and diagnostic methods, in addition to devices to carry out these methods. The differentially expressed genes include both "fingerprint genes" and "control genes." "Fingerprint genes" are those nucleic acids that correlate with a particular type of lymphocyte (*e.g.*, Th1 or Th2), or a particular cellular state (*e.g.*, activated or non-activated). As described in greater detail below, fingerprint genes can be used in the development of a variety of different screening and diagnostic methods to classify types of lymphocytes and/or to aid in the diagnosis of particular disease conditions. A "control gene" is one that encodes a protein that is involved in a lymphocyte assuming a particular state or becoming a particular type of cell. Because of the role such genes play, control genes are useful targets for the development of

compound discovery programs and pharmaceutical development such as described *infra*. In some instances, a fingerprint gene can be a control gene and vice versa.

Expression levels for combinations of differentially expressed genes, in particular fingerprint genes, can be used to develop "expression profiles" that are characteristic of a particular type of lymphocyte or cellular state. Expression profiles as used herein refers to the pattern of gene expression corresponding to at least one differentially expressed genes, but typically includes a plurality of genes. For instance, an expression profile can include at least 1, 2, 3, 4 or 5 differentially expressed genes, but in other instances can include at least 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 45 or 50 or more differentially expressed genes. In some instances, expression profiles include all of the differentially expressed genes known for a particular type of lymphocyte or cellular state. So, for example, certain expression profiles include a measure (quantitative or qualitative) of the expression level for each of the differentially expressed genes in one or more of the tables or figures (*e.g.*, Table 1 or Table 2 and/or Table6).

The pattern of expression associated with gene expression profiles can be defined in several ways. For example, a gene expression profile can be the absolute (*e.g.*, a measured value) or relative transcript level of any number of particular differentially expressed genes. In other instances, a gene expression profile can be defined by comparing the level of expression of a variety of genes in one state to the level of expression of the same genes in another state (*e.g.*, activated versus unactivated), or between one cell type and another cell type (*e.g.*, Th1 versus Th2).

As used herein, the term "differentially expressed gene" or "differentially expressed nucleic acid" refers to the specific sequence as set forth in the particular GenBank entry that is provided herein (*see, e.g.*, the tables and figures). The term, however, is also intended to include more broadly naturally occurring sequences (including allelic variants of those listed for the GenBank entries), as well as synthetic and intentionally manipulated sequences (*e.g.*, nucleic acids subjected to site-directed mutagenesis). It is noted that the sequences of the target genes listed in the tables and figures are available in the public databases. The tables provide the accession number and name for each of the sequences. The sequences of the genes in GenBank are herein expressly incorporated by reference in their entirety as of the filing date of this application (see www.ncbi.nim.nih.gov).

Differentially expressed nucleic acids also include sequences that are complementary to the listed sequences, as well as degenerate sequences resulting from the

degeneracy of the genetic code. Thus, the differentially expressed nucleic acids include: (a) nucleic acids having sequences corresponding to the sequences as provided in the listed GenBank accession number; (b) nucleic acids that encode amino acids encoded by the nucleic acids of (a); (c) a nucleic acid that hybridizes under stringent conditions to a complement of the nucleic acid of (a); and (d) nucleic acids that hybridize under stringent conditions to, and therefore are complements of, the nucleic acids described in (a) through (c). The differentially expressed nucleic acids of the invention also include: (a) a deoxyribonucleotide sequence complementary to the full-length nucleotide sequences corresponding to the listed GenBank accession numbers; (b) a ribonucleotide sequence complementary to the full-length sequence corresponding to the listed GenBank accession numbers; and (c) a nucleotide sequence complementary to the deoxyribonucleotide sequence of (a) and the ribonucleotide sequence of (b). The differentially expressed nucleic acids further include fragments of the foregoing sequences. For example, nucleic acids including 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, 225, 250, 275 or 300 contiguous nucleotides (or any number of nucleotides therebetween) from a differentially expressed nucleic acid are included. Such fragments are useful, for example, as primers and probes for hybridizing full-length differentially expressed nucleic acids (e.g., in detecting and amplifying such sequences).

In some instances, the differentially expressed nucleic acids include conservatively modified variations. Thus, for example, in some instances, the differentially expressed nucleic acids are modified. One of skill will recognize many ways of generating alterations in a given nucleic acid construct. Such well-known methods include site-directed mutagenesis, PCR amplification using degenerate polynucleotides, exposure of cells containing the nucleic acid to mutagenic agents or radiation and chemical synthesis of a desired polynucleotide (*e.g.*, in conjunction with ligation and/or cloning to generate large nucleic acids). *See, e.g.*, Giliman and Smith (1979) *Gene* 8:81-97, Roberts *et al.* (1987) *Nature* 328: 731-734). When the differentially expressed nucleic acids are incorporated into vectors, the nucleic acids can be combined with other sequences including, but not limited to, promoters, polyadenylation signals, restriction enzyme sites and multiple cloning sites. Thus, the overall length of the nucleic acid can vary considerably.

Certain differentially expressed nucleic acids of the invention include polynucleotides that are substantially identical to a polynucleotide sequence as set forth in SEQ ID NO:1. Such nucleic acids can function as new markers for certain types of

lymphocytes and for different cellular states for lymphocytes. For example, the invention includes polynucleotide sequences that are at least 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% identical to the polynucleotide sequences provided in the GenBank entries listed in the tables. Identity is typically measured over at least 40, 50, 60, 70, 80, 90 or 100 contiguous nucleotides. In other instances, identity is measured over a region of at least 150, 200, or 250 nucleotides in length. In yet other instances, the region of similarity exceeds 250 nucleotides in length and extends for at least 300, 350, 400, 450 or 500 nucleotides in length, or over the entire length of the sequence.

As described above, sequence identity comparisons can be conducted using a nucleotide sequence comparison algorithm such as those know to those of skill in the art. For example, one can use the BLASTN algorithm. Suitable parameters for use in BLASTN are wordlength (W) of 11, M=5 and N=-4 and the identity values and region sizes just described.

B. Preparation of Differentially Expressed Genes

The differentially expressed nucleic acids can be obtained by any suitable method known in the art, including, for example: (1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide sequences; (2) antibody screening of expression libraries to detect cloned DNA fragments with shared structural features; (3) various amplification procedures such as polymerase chain reaction (PCR) using primers capable of annealing to the nucleic acid of interest; and (4) direct chemical synthesis.

The desired nucleic acids can also be cloned using well-known amplification techniques. Examples of protocols sufficient to direct persons of skill through *in vitro* amplification methods, including the polymerase chain reaction (PCR) the ligase chain reaction (LCR), Qβ-replicase amplification and other RNA polymerase mediated techniques, are found in Berger, Sambrook, and Ausubel, as well as Mullis *et al.* (1987) U.S. Patent No. 4,683,202; *PCR Protocols A Guide to Methods and Applications* (Innis *et al.* eds) Academic Press Inc. San Diego, CA (1990) (Innis); Arnheim & Levinson (October 1, 1990) *C&EN* 36-47; *The Journal Of NIH Research* (1991) 3: 81-94; (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86: 1173; Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87: 1874; Lomell *et al.* (1989) *J. Clin. Chem.* 35: 1826; Landegren *et al.* (1988) *Science* 241: 1077-1080; Van Brunt (1990) *Biotechnology* 8: 291-294; Wu and Wallace (1989) *Gene* 4: 560; and Barringer *et al.* (1990) *Gene* 89: 117. Improved methods of

cloning *in vitro* amplified nucleic acids are described in Wallace *et al.*, U.S. Pat. No. 5,426,039.

As an alternative to cloning a nucleic acid, a suitable nucleic acid can be chemically synthesized. Direct chemical synthesis methods include, for example, the phosphotriester method of Narang *et al.* (1979) *Meth. Enzymol.* 68: 90-99; the phosphodiester method of Brown *et al.* (1979) *Meth. Enzymol.* 68: 109-151; the diethylphosphoramidite method of Beaucage *et al.* (1981) *Tetra. Lett.*, 22: 1859-1862; and the solid support method described in U.S. Patent No. 4,458,066. Chemical synthesis produces a single stranded polynucleotide. This can be converted into double stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. While chemical synthesis of DNA is often limited to sequences of about 100 bases, longer sequences can be obtained by the ligation of shorter sequences. Alternatively, subsequences can be cloned and the appropriate subsequences cleaved using appropriate restriction enzymes. The fragments can then be ligated to produce the desired DNA sequence.

C. Utility of Differentially Expressed Nucleic Acids and Expression Profiles

As alluded to above and described in greater detail below, the differentially expressed nucleic acids that are provided can be used as markers in a variety of screening and diagnostic methods. For example, the differentially expressed nucleic acids find utility as hybridization probes or amplification primers. In certain instances, these probes and primers are fragments of the differentially expressed nucleic acids of the lengths described earlier in this section. Such fragments are generally of sufficient length to specifically hybridize to an RNA or DNA in a sample obtained from a subject. The nucleic acids are typically 10-30 nucleotides in length, although they can be longer as described above. The probes can be used in a variety of different types of hybridization experiments, including, but not limited to, Northern blots and Southern blots and in the preparation of custom arrays (see *infra*). The differentially expressed nucleic acids can also be used in the design of primers for amplifying the differentially expressed nucleic acids and in the design of primers and probes for quantitative RT-PCR. The primers most frequently include about 20 to 30 contiguous nucleotides of the differentially expressed nucleic acids to obtain the desired level of stability and thus selectivity in amplification, although longer sequences as described above can also be utilized.

Hybridization conditions are varied according to the particular application. For applications requiring high selectivity (*e.g.*, amplification of a particular sequence), relatively stringent conditions are utilized, such as 0.02 M to about 0.10 M NaCl at temperatures of about 50 °C to about 70 °C. High stringency conditions such as these tolerate little, if any, mismatch between the probe and the template or target strand of the differentially expressed nucleic acid. Such conditions are useful for isolating specific genes or detecting particular mRNA transcripts, for example.

Other applications, such as substitution of amino acids by site-directed mutagenesis, require less stringency. Under these conditions, hybridization can occur even though the sequences of the probe and target nucleic acid are not perfectly complementary, but instead include one or more mismatches. Conditions can be rendered less stringent by increasing the salt concentration and decreasing temperature. For example, a medium stringency condition includes about 0.1 to 0.25 M NaCl at temperatures of about 37 °C to about 55 °C. Low stringency conditions include about 0.15M to about 0.9 M salt, at temperatures ranging from about 20 °C to about 55 °C.

V. Proteins

A. General

The differentially expressed nucleic acids that have been identified can be inserted into any of a number of known expression systems to generate large amounts of the protein encoded by the gene or gene fragment. Such proteins can then be utilized in the preparation of antibodies. Proteins encoded by target genes can be utilized in the compound development programs described below and in the preparation of various diagnostics (e.g., antibody arrays).

The polypeptides can be isolated from natural sources, and/or prepared according to recombinant methods, and/or prepared by chemical synthesis, and/or prepared using a combination of recombinant methods and chemical synthesis. Besides substantially full-length polypeptides, biologically active fragments of the polypeptides are also provided. Biological activity can include, for example, antibody binding (*e.g.*, the fragment competes with a full-length polypeptide) and immunogenicity (*i.e.*, possession of epitopes that stimulate B- or T-cell responses against the fragment). Such fragments generally comprise at least 5 contiguous amino acids, typically at least 6 or 7 contiguous amino acids, in other instances 8 or 9 contiguous amino acids, usually at least 10, 11 or 12 contiguous amino acids, in still other instances at least 13 or 14 contiguous amino acids, in yet other instances

at least 16 contiguous amino acids, and in some cases at least 20, 40, 60 or 80 contiguous amino acids.

Often the polypeptides will share at least one antigenic determinant in common with the amino acid sequence of the full-length polypeptide. The existence of such a common determinant is evidenced by cross-reactivity of the variant protein with any antibody prepared against the full-length polypeptide. Cross-reactivity can be tested using polyclonal sera against the full-length polypeptide, but can also be tested using one or more monoclonal antibodies against the full-length polypeptide.

The polypeptides include conservative variations of the naturally occurring polypeptides. Such variations can be minor sequence variations of the polypeptide that arise due to natural variation within the population (*e.g.,* single nucleotide polymorphisms) or they can be homologs found in other species. They also can be sequences that do not occur naturally but that are sufficiently similar so that they function similarly and/or elicit an immune response that cross-reacts with natural forms of the polypeptide. Sequence variants can be prepared by standard site-directed mutagenesis techniques. The polypeptide variants can be substitutional, insertional or deletion variants. Deletion variants lack one or more residues of the native protein that are not essential for function or immunogenic activity (*e.g.*, polypeptides lacking transmembrane or secretory signal sequences). Substitutional variants involve conservative substitutions of one amino acid residue for another at one or more sites within the protein and can be designed to modulate one or more properties of the polypeptide such as stability against proteolytic cleavage. Insertional variants include, for example, fusion proteins such as those used to allow rapid purification of the polypeptide and also can include hybrid proteins containing sequences from other polypeptides, which are homologues of the polypeptide. The foregoing variations can be utilized to create equivalent, or even an improved, second-generation polypeptide. Preparation of variants is well known in the art (see, e.g., Creighton (1984) *Proteins*, W.H. Freeman and Company, which is incorporated herein by reference in its entirety for all purposes).

The polypeptides that are provided also include those in which the polypeptide has a modified polypeptide backbone. Examples of such modifications include chemical derivatizations of polypeptides, such as acetylations and carboxylations. Modifications also include glycosylation modifications and processing variants of a typical polypeptide. Such processing steps specifically include enzymatic modifications, such as ubiquitinization and phosphorylation. *See, e.g.*, Hershko & Ciechanover, *Ann. Rev.*

Biochem. 51:335-364 (1982). Also included are mimetics, which are peptide-containing molecules that mimic elements of protein secondary structure (see, *e.g.*, Johnson, *et al.*, "Peptide Turn Mimetics" in *Biotechnology and Pharmacy*, (Pezzuto et al., Eds.), Chapman and Hall, New York (1993)). Peptide mimetics are typically designed so that side chain groups extending from the backbone are oriented such that the side chains of the mimetic can be involved in molecular interactions similar to the interactions of the side chains in the native protein.

B. Production of Polypeptides

1. Recombinant Technologies

The polypeptides encoded by the differentially expressed nucleic acids can be expressed in hosts after the coding sequences have been operably linked to an expression control sequence in an expression vector. Expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Expression vectors commonly contain selection markers, *e.g.*, tetracycline resistance or hygromycin resistance, to permit detection and/or selection of those cells transformed with the desired DNA sequences (*see, e.g.*, U.S. Patent 4,704,362).

A differentially expressed gene typically is placed under the control of a promoter that is functional in the desired host cell to produce relatively large quantities of a polypeptide of the invention. An extremely wide variety of promoters are well known to those of skill, and can be used in the expression vectors, depending on the particular application. Ordinarily, the promoter selected depends upon the cell in which the promoter is to be active. Other expression control sequences such as ribosome binding sites, transcription termination sites and the like are also optionally included. Constructs that include one or more of such control sequences are termed "expression cassettes." Accordingly, expression cassettes are provided into which the differentially expressed nucleic acids are incorporated for high level expression of the corresponding protein in a desired host cell.

A wide variety of cloning and *in vitro* amplification methods suitable for the construction of recombinant nucleic acids is described, for example, in Berger and Kimmel, *Guide to Molecular Cloning Techniques, Methods in Enzymology*, Volume 152, Academic Press, Inc., San Diego, CA (Berger); and "Current Protocols in Molecular Biology," F.M. Ausubel *et al.*, eds., *Current Protocols*, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1998 Supplement) (Ausubel).

2. Naturally Occurring Polypeptides

Naturally occurring polypeptides encoded by the differentially expressed genes can also be isolated using conventional techniques such as affinity chromatography. For example, polyclonal or monoclonal antibodies can be raised against the polypeptide of interest and attached to a suitable affinity column by well-known techniques. *See, e.g.*, Hudson & Hay, *Practical Immunology* (Blackwell Scientific Publications, Oxford, UK, 1980), Chapter 8 (incorporated by reference in its entirety). Peptide fragments can be generated from intact polypeptides by chemical or enzymatic cleavage methods known to those of skill in the art.

3. Other Methods

Alternatively, the polypeptides encoded by differentially expressed genes or gene fragments can be synthesized by chemical methods or produced by *in vitro* translation systems using a polynucleotide template to direct translation. Methods for chemical synthesis of polypeptides and *in vitro* translation are well-known in the art, and are described further by Berger & Kimmel, *Methods in Enzymology, Volume 152, Guide to Molecular Cloning Techniques*, Academic Press, Inc., San Diego, CA, 1987 (incorporated by reference in its entirety).

C. Utility

The polypeptides can be used to generate antibodies that specifically bind to epitopes associated with the polypeptides or fragments thereof. Commercially available computer sequence analysis can be used to determine the location of the predicted major antigenic determinant epitopes of the polypeptide (*e.g.*, MacVector from IBI, New Haven, Conn.). Once such an analysis has been performed, polypeptides can be prepared that contain at least the essential structural features of the antigenic determinant and can be utilized in the production of antisera against the polypeptide. Minigenes or gene fusions encoding these determinants can be constructed and inserted into expression vectors such as those described above using standard techniques. The major antigenic determinants can also be determined empirically in which portions of the gene encoding the polypeptide are expressed in a recombinant host, and the resulting proteins tested for their ability to elicit an immune response. For example, PCR can be used to prepare a range of cDNAs encoding polypeptides lacking successively longer fragments of the C-terminus of the polypeptide. The immunoprotective activity of each of these polypeptides then identifies those fragments or domains of the polypeptide that are essential for this activity. Further

experiments in which only a small number or amino acids are removed at each iteration then allows the location of the antigenic determinants of the polypeptide.

Polypeptides encoded by target genes can be utilized in the development of pharmaceutical compositions, for example that modulate gene products associated cancerous cells. The process for identifying such polypeptides and subsequent compound development is described further below.

VI. Exemplary Screening, Diagnostic and Classification Methods

A. General Considerations

Certain methods that are provided involve determining the expression level of one or more of the differentially expressed genes in a test cell population with the expression level of the same genes in a control cell population, or comparing the expression profile for one sample with an expression profile determined for another sample. The level of expression of the differentially expressed nucleic acids can be determined at either the nucleic acid level or the protein level. Thus, the phrase "determining the expression level," "preparing a gene expression profile," and other like phrases when used in reference to the differentially expressed nucleic acids means that transcript levels and/or levels of protein encoded by the differentially encoded nucleic acids are detected. When determining the level of expression, the level can be determined qualitatively, but generally is determined quantitatively.

Based upon the sequence information that is disclosed herein, coupled with the nucleic acid and protein detection methods that are described herein and that are known in the art, expression levels of these genes can readily determined. If transcript levels are determined, they can be determined using routine methods. For instance, the sequence information provided herein (e.g., GenBank sequence entries) can be used to construct nucleic acid probes using conventional methods such as various hybridization detection methods (e.g., Northern blots). Alternatively, the provided sequence information can be used to generate primers that in turn are used to amplify and detect differentially expressed nucleic acids that are present in a sample (e.g., quantitative RT-PCR methods). If instead expression is detected at the protein level, encoded protein can be detected and optionally quantified using any of a number of established techniques. One common approach is to use antibodies that specifically bind to the protein product in immunoassay methods. Additional details regarding methods of conducting differential gene expression are provided infra.

Expression levels can be detected for one, some, or all of the differentially expressed nucleic acids that are listed in one or more of the tables (*e.g.*, Table 1, or Table 2, or Table 6). With some methods, the expression levels for only 1, 2, 3, 4 or 5 differentially expressed nucleic acids are determined. In other methods, expression levels for at least 6, 7, 8, 9 or 10 differentially expressed nucleic acids are determined. In still other methods, expression levels for at least 15, 20, 25, 30, 35, 40, 45, 50, 55, or 60 differentially expressed nucleic acids are determined. In yet other methods, all of the differentially expressed genes in one or more of the tables are determined.

Determination of expression levels is typically done with a test sample taken from a test cell population. As used herein, the term "population" when used in reference to a cell can mean a single cell, but typically refers to a plurality of cells (e.g., a tissue sample). Certain screening methods are performed with test cells that are "capable of expressing" one or more of the differentially expressed nucleic acids. As used in this context, the phrase "capable or expressing" means that the gene of interest is in intact form and can be expressed within the cell.

A number of the methods that are provided involve a comparison of expression levels for certain differentially expressed nucleic acids in a "test cell" with the expression levels for the same nucleic acids in a "control cell" (also sometimes referred to as a "control sample," a "reference cell," a "reference value," or simply a "control"). Other methods involve a comparison between one expression profile and a baseline expression profile. In either case, the expression level for the control cell or baseline expression profile essentially establishes a baseline against which an experimental value is compared. The comparison of expression levels are meant to be interpreted broadly with respect to what is meant by: 1) the term "cell", 2) the time at which the expression levels for test and control cells are determined, and 3) with respect to the measure of the expression levels.

So, for example, although the term "test cell" and "control cell" is used for convenience, the term "cell" is meant to be construed broadly. A cell, for instance, can also refer to a population of cells (e.g., a tissue sample), just as a population of cells can have a single member. The cell may in some instances be a sample that is derived from a cell (e.g., a cell lysate, a homogenate, or a cell fraction). In general samples can be obtained from various sources, particularly sources of lymphocytes.

With respect to timing, comparison of expression levels can be done contemporaneously (e.g., a test and control cell are each contacted with a test agent in parallel reactions). The comparison alternatively can be conducted with expression levels

that have been determined at temporally distinct times. As an example, expression levels for the control cell can be collected prior to the expression levels for the test cell and stored for future use (e.g., expression levels stored on a computer compatible storage medium).

The expression level for a control cell or baseline expression profile (e.g., baseline value) can be a value for a single cell or it can be an average, mean or other statistical value determined for a plurality of cells. As an example, the expression level for a control cell can be the average of the expression levels for a population of subjects (e.g., subjects not having an immune-related disorder such as asthma). In other instances, the value for each expression level for the control cell is a range of values representative of the range observed for a particular population. Expression level values can also be either qualitative or quantitative. The values for expression levels can also optionally be normalized with respect to the expression level of a nucleic acid that is not one of the markers under analysis.

The comparative analysis required in some methods involves determining whether the expression level values are "comparable" (or similar"), or "differ" from one another. In some instances, the expression levels for a particular marker in test and control cells are considered similar if they differ from one another by no more than the level of experimental error. Often, however, expression levels are considered similar if the level in the test cell differs by less than 5%, 10%, 20%, 50%, 100%, 150%, or 200% with respect to the control cell,. It thus follows that in some instances the expression level for a particular marker in the test cell is considered to differ from the expression level for the same marker in the control cell if the difference is greater than the level of experimental error, or if it is greater than 5%, 10%, 20%, 50%, 100%, 150% or 200%. In some methods, the comparison involves a determination of whether there is a "statistically significant difference" in the expression level for a marker in the test and control cells. A difference is generally considered to be "statistically significant" if the probability of the observed difference occurring by chance (the p-value) is less than some predetermined level. As used herein a "statistically significant difference" refers to a p-value that is < 0.05, preferably < 0.01 and most preferably < 0.001. If gene expression is increased sufficiently such that it is different (as just defined) relative to the control cell or baseline, the expression of that gene is considered "up-regulated" or "increased." If, instead, gene expression is decreased so it differs from the control cell or baseline value, the expression of that gene is "down-regulated" or "decreased."

Comparison of the expression levels between test and control cells can involve comparing levels for a single marker or a plurality of markers (e.g., when expression profiles are compared). When the expression level for a single marker is determined, whether expression levels between the test and control cell are similar or different involves a comparison of the expression level of the single marker. When, however, expression levels for multiple markers are compared, the comparison analysis can involve two analyses: 1) a determination for each marker examined whether the expression level is similar between the test and control cells, and 2) a determination of how many markers from the group of markers examined show similar or different expression levels. The first determination is done as just described. The second determination typically involves determining whether at least 50% of the markers examined show similarity in expression levels. However, in methods were more stringent correlations are required, at least 60%, 70%, 80%, 90%, 95% or 100% of the markers must show similar expression levels for the expression levels of the group of markers examined considered to be similar between the test and control cells.

B. Screening Methods

1. Exemplary Approaches

Monitoring changes in gene expression can provide certain advantages during drug screening and development. Often drugs are pre-screened for the ability to interact with a major target without regard to other effects the drugs have on cells. Often such other effects cause toxicity in the whole animal, which prevent the development and use of the potential drug. These global changes in gene expression provide useful markers for diagnostic uses as well as markers that can be used to monitor disease states, disease progression, and drug metabolism. Thus, these expression profiles of genes provide molecular tools for evaluating drug toxicity, drug efficacy, and disease monitoring.

Changes in the expression profile from a baseline profile (*e.g.*, the data in Table 1) can be used as an indication of such effects. Those skilled in the art can use any of a variety of known techniques to evaluate the expression of one or more of the genes and/or gene fragments identified in the present application in order to observe changes in the expression profile in a cell or sample of interest. Comparison of the expression data, as well as available sequence or other information may be done by researcher or diagnostician or may be done with the aid of a computer and databases.

In some screening methods, compounds and molecules are screened to identify those that affect expression of a target gene or some other gene involved in regulating the expression of a target gene (*e.g.*, by interacting with the regulatory region or transcription factors of a target gene). Compounds are also screened to identify those that affect the activity of such proteins (*e.g.*, by inhibiting target gene activity) or the activity of a molecule involved in the regulation of a target gene.

So, for example, in some methods potential drug compounds are screened to determine if application of the compound alters the expression of one or more of the target genes identified herein. This may be useful, for example, in determining whether a particular compound is effective in treating asthma or other immune-mediated disease. In the case in which the expression of a gene during the CD4+ lymphocyte polarization is affected by the potential drug compound, the compound is indicated in the treatment of asthma or other immune-mediated disease. Similarly, a drug compound which causes expression of a gene which is normally down-regulated during the CD4+ lymphocyte polarization, may be indicated in the treatment of the same diseases.

According to the present invention, the target genes listed in Table 2 or Table 6 may also be used as markers to evaluate the effects of a candidate drug or agent on a lymphocyte cell, particularly undergoing polarization. A candidate drug or agent can be screened for the ability to stimulate the transcription or expression of a given marker or markers (drug targets) or to down-regulate or inhibit the transcription or expression of a marker or markers. According to the present invention, one can also compare the specificity of a drug's effects by looking at the number of markers affected by the drug and comparing them to the number of markers affected by a different drug. A more specific drug will affect fewer transcriptional targets. Similar sets of markers identified for two drugs indicates a similarity in effect.

Some method are designed for identifying agents that modulate the levels, concentration or at least one activity of a protein(s) encoded by one or several genes in Table 2 or Table 6. Such methods or assays may utilize any means of monitoring or detecting the desired activity.

One specific embodiment of the invention is a method of identifying a compound capable of modulating the polarization of CD4+ lymphocytes, the method comprising:

(a) contacting the compound with naïve CD4+ lymphocytes;

(b) inducing the polarization of the lymphocytes;

(c) preparing a gene expression profile from the lymphocytes;

(d) comparing the lymphocyte gene expression profile to a gene expression profile derived from Table 1.

Preferably the induction of step (b) is performed by contacting the lymphocytes with a cytokine. Preferably the cytokine is IL-12 or IL-4. A difference in the expression profiles of the target genes identifies a potential drug compound for the treatment of asthma or other immune-mediated diseases. Another preferred embodiment of the invention is an identification method of the invention, wherein said gene expression profile derived from Table 1 is at least partly based on the expression fold changes of any one of the genes selected from the group consisting of: KIAA0053, LRRN3, CIG5, DUSP6, FER1L3, S100P, SATB1, SLC11A2, STK17B, a gene identified by accession number AI971169 and a gene identified by accession number AL432401.

Assays and screens can be used to identify compounds that are effective activators or inhibitors of target gene expression or activity. The assays and screens can be done by physical selection of molecules from libraries, and computer comparisons of digital models of compounds in molecular libraries and a digital model of the active site of the target gene product (*i.e.*, protein).

The activators or inhibitors identified in the assays and screens may act by, but are not limited to, binding to a target gene product, binding to intracellular proteins that bind to a target gene product, compounds that interfere with the interaction between a target gene product and its substrates, compounds that modulate the activity of a target gene, or compounds that modulate the expression of a target gene or a target gene product.

Assays can also be used to identify molecules that bind to target gene regulatory sequences (*e.g.*, promoter sequences), thus modulating gene expression. See, *e.g.*, Platt (1994), J. Biol. Chem., 269:28558-28562.

Another specific embodiment of the invention is a method of identifying a compound that modulates the expression of at least one gene listed in Table 2 or Table 6. These methods involve:

(a) incubating a cell that can express a protein from the gene with a compound under conditions and for a time sufficient for the cell to express the protein of said gene, when the compound is not present;

(b) incubating a control cell under the same conditions and for the same time without the compound;

(c) measuring expression of the gene in the cell in the presence of the compound;

(d) measuring expression of the gene in the control cell; and

(e) comparing the amount of expression of the gene in the presence and absence of the compound, wherein a difference in the level of expression indicates that the compound modulates the expression of the gene.

Another specific embodiment of the invention is a method of identifying a compound that modulates the activity of at least one gene listed in Table 2 or Table 6, the method comprising:

(a) incubating a cell that has said activity with a compound under conditions and for a time sufficient for the cell to express said activity, when the compound is not present;

(b) incubating a control cell under the same conditions and for the same time without the compound;

(c) measuring said activity in the cell in the presence of the compound;

(d) measuring said activity in the control cell; and

(e) comparing the amount of said activity in the presence and absence of the compound, wherein a difference in the level of expression indicates that the compound modulates the activity of said gene.

In one preferred embodiment, the gene or genes is/are selected from the group consisting of: KIAA0053, LRRN3, CIG5, DUSP6, FER1L3, S100P, SATB1, SLC11A2, STK17B, a gene identified by accession number AI971169 and a gene identified by accession number AL432401.

Certain screening methods are performed with mouse lymphocytes. As an example, some methods involve identifying a compound capable of modulating the polarization of murine CD4+ lymphocytes. These methods comprise:

(a) contacting the compound with naïve murine CD4+ lymphocytes;

(b) inducing the polarization of the lymphocytes;

(c) preparing a gene expression profile from the lymphocytes; and

(d) comparing the lymphocyte gene expression profile to a gene expression profile derived from Table 3.

This method can also be used for determining or confirming the activity of a compound in murine cells, when said compound is already identified in any one of the above-mentioned human lymphocyte assay based identification methods as a modulator for polarization of human lymphocytes.

2. Methods for Detecting Differential Gene Expression

Assays to monitor the expression of a marker or markers as defined in Table 2 or Table 6 may utilize any available means of monitoring for changes in the expression level of the target genes. As used herein, an agent is said to modulate the expression of a target gene if it is capable of up- or down-regulating expression of the target gene in a lymphocyte cell during polarization. The protein products encoded by the genes identified herein can also be assayed to determine the amount of expression. Any method for specifically and quantitatively measuring a specific protein or mRNA or DNA product can be used. However, methods and assays of the invention typically utilize PCR or array or chip hybridization-based methods when seeking to detect the expression of a large number of genes.

[0001] The genes identified as being differentially expressed in lymphocytes may be used in a variety of nucleic acid detection assays to detect or quantify the expression level of a gene or multiple genes in a given sample. For example, traditional Northern blotting, dot blots, nuclease protection, RT-PCR, differential display methods, subtractive hybridization, and in situ hybridization may be used for detecting gene expression levels. Levels of mRNA expression may be monitored directly by hybridization of probes to the nucleic acids of the invention. Cell lines are exposed to an agent to be tested under appropriate conditions and time and total RNA or mRNA is isolated by standard procedures such those disclosed in Sambrook et al, Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989).

One of skill in the art will appreciate that an enormous number of array designs are suitable for the practice of this invention. The high density array will typically include a number of probes that specifically hybridize to the sequences of interest. See WO 99/32660 for methods of producing probes for a given gene or genes. In addition, in a preferred embodiment, the array will include one or more control probes.

3. Exemplary Candidate Agents

Agents that are assayed in the above methods can be randomly selected or rationally selected or designed. As used herein, an agent is said to be randomly selected when the agent is chosen randomly without considering the specific sequences of the protein itself or those sequences involve din the interaction of the protein with its substrates or ligands, for instance. An example of randomly selected agents is a chemical library or a peptide combinatorial library, or a growth broth of an organism. As used herein, an agent is said to be rationally selected or designed when the agent is chosen on a

nonrandom basis, taking into account the sequence of the target site and/or its conformation in connection with the agents action. Agents can be selected or designed by utilizing the peptide sequences that make up these sites. For example, a rationally selected peptide agent can be a peptide whose amino acid sequence is identical to or a derivative of any functional consensus site.

The agents of the present invention can be, as examples, peptides, small chemical molecules, vitamin derivatives, as well as carbohydrates, lipids, oligonucleotides and covalent and non-covalent combinations thereof. Dominant negative proteins, DNA encoding these proteins, antibodies to these proteins, peptide fragments of these proteins or mimics of these proteins may be introduced into cells. A "mimetic" as used herein refers to a protein having a modification of a region or several regions such that the protein mimetic has a structure chemically different from the parent peptide but topographically and functionally similar to the parent peptide. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents of the present invention.

Some compounds are peptides, including but not limited to, soluble peptides, including but not limited to members of random peptide libraries (see, *e.g.*, Lam et al. (1991) Nature 354:82-84; Houghten et al. (1991) Nature 354:84-86,), and combinatorial chemistry-derived molecular libraries made of D-and/or L-amino acids, phosphopeptides (including, but not limited to, members of random or partially degenerate, directed phosphopeptide libraries; see, *e.g.*, Songyang et al. (1993) Cell 72:767-778,), and small organic or inorganic molecules.

3. Computerized Analysis

Computer modeling or searching technologies can be used to identify compounds, or identify modified compounds that modulate or are candidates to modulate the expression or activity of a target gene product. For example, compounds likely to interact with the active site of the target gene product may be identified. The active site of target gene product can be identified using methods known in the art including, for example, analysis of the amino acid sequence of a molecule, and from a study of complexes formed by a target gene product with a native ligand. Chemical or X-ray crystallographic methods can be used to identify the active site of target gene product through the location of a bound ligand.

The three-dimensional structure of the active site can be determined. This can be done using known methods, including X-ray crystallography, which can be used to

determine a complete molecular structure. Solid or liquid phase NMR can be used to determine certain intramolecular distances. Other methods of structural analysis can be used to determine partial or complete geometrical structures.

Computer-based numerical modeling can be used to complete an incomplete or insufficiently accurate structure. Modeling methods that can be used are, for example, parameterized models specific to particular biopolymers such as proteins or nucleic acids, molecular dynamics models based on computing molecular motions, statistical mechanics models based on thermal ensembles, or combined models. For most types of models, standard molecular force fields, representing the forces between constituent atoms and groups are necessary, and can be selected from force fields known in physical chemistry. Information on incomplete or less accurate structures determined in this way can be incorporated as constraints on the structures computed by these modeling methods.

Having determined the structure of the active site of a target gene product, either experimentally, by modeling, or by a combination of methods, additional candidate modulating compounds can be identified by searching databases containing compounds along with information on their molecular structure. The compounds identified in such a search are those that have structures that match the active site structure, fit into the active site, or interact with groups defining the active site. The compounds identified by the search are potential target gene product modulating compounds.

These methods may also be used to identify improved modulating compounds from an already known modulating compounds or ligands. The structure of the known compound is modified and effects are determined using experimental and computer modeling methods. The altered structure is compared to the active site structure of a target gene product to determine or predict how a particular modification to the ligand or modulating compound will affect its interaction with that protein. Systematic variations in composition, such as by varying side groups, can be evaluated to obtain modified modulating compounds or ligands of preferred specificity or activity.

Given the teachings herein, additional experimental and computer modeling methods useful to identify modulating compounds based on identification of the active sites of a target gene product and related transduction and transcription factors can be developed by those skilled in the art. Computer programs designed to screen and depict chemicals as well as molecular modeling systems are available from companies such as MSI (Molecular Simulations, Inc., San Diego, CA, USA), Allelix, Inc. (Mississauga, Ontario, Canada), and Hypercube, Inc. (Gainesville, FL, USA).

In addition to designing and generating compounds that alter binding, as described above, libraries of known compounds, including natural products, synthetic chemicals, and biologically active materials including peptides, can be screened for compounds that are inhibitors or activators.

Compounds identified by methods described above may be useful, for example, for elaborating the biological function of target gene products and in treatment of disorders in which target gene activity is deleterious.

C. Diagnostic Methods

Methods for assessing whether a subject has or is predisposed to obtain an immune-mediated disease (*e.g.* asthma, allergy or auto-immune disease) are also provided. These methods generally involve obtaining a sample from a subject having or suspected to have an immune-mediated disease. The expression levels for one or more the differentially expressed genes is then determined for the sample. The population of test cells is selected to include lymphocytic cells from the subject.

The expression level of the gene(s) is then compared with the expression level of the same gene(s) in a control sample. The status of the control sample with respect to presence or absence of an immune-mediated disease is known (e.g., the control sample is from an individual not suffering from the immune-related disease of interest or is from an individual having an immune-related disease). So, for example, if the control cell is representative of cells from a healthy individual, then similarity in expression level or expression profile between the test and control samples indicates that the subject does not have an immune-related disease. A difference in expression level or profile, in contrast, indicates that the subject from whom the test sample was derived has an immune-related disease.

If instead, the control sample is representative of cells from an individual that has a particular immune-related disease, then similarity in expression levels or expression profile means that the test cells are from a patient that has, or is susceptible to, the immune-related disease; whereas, a difference in expression levels or profile indicates that the subject does not have the immune-related disease.

D. Classifying Lymphocytes

Other methods that are provided are designed to classify a lymphocyte or to assess its cellular state. Such methods generally involve obtaining a test sample derived from a lymphocyte that is capable of expressing one or more nucleic acid markers from the

group consisting of those listed in one or more of the tables (*e.g.*, Table 1, or Table 2 or Table 6. The expression level for one or more of these markers is then determined. The expression level for these markers is compared with the expression levels of the same markers in a control sample. The control sample is derived from a lymphocytic cell whose cellular status is known (*e.g.*, the cell is known to be a Th1 or Th2 cell). The lymphocytic cell from which the test sample is derived is then classified on the basis of this comparison.

For example, if the expression level or expression profile of the test sample is compared with expression levels or an expression profile from a Th1 cell, similarity in expression profile is an indication that the lymphocyte from which the test sample is derived is a Th1 cell. A difference, on the other hand, would be an indication that the sample is from a lymphocyte of another type or in another state (e.g., a Th2 cell).

VII. Treatment Methods

Methods of treating a patient with asthma or other immune-mediated diseases are also provided. These methods generally involve administering to the patient a pharmaceutical composition, wherein the composition alters the expression or activity of at least one gene listed in Table 2 or Table 6. In a preferred embodiment, the active compound of the pharmaceutical composition is identified by a screening method of the invention. In another preferred embodiment, the active compound of said pharmaceutical composition is an antibody binding to at least one gene product of the genes listed in Table 2 or Table 6.

Both therapeutic and prophylactic methods are provided. In therapeutic methods, a pharmaceutical composition is administered to a subject having or suspected to have an immune-related disease in an amount sufficient to alleviate one or more symptoms of the disease. In prophylactic methods, a pharmaceutical composition is administered to a subject susceptible to, or otherwise at risk for developing an immune-related disease, in an amount sufficient to reduce or arrest the development of the disease. The treatment can be administered in a single dose, but more commonly is administered in several doses.

If the immune-related disease, is a consequence of an excessive Th1 response, then certain methods involve administering an agent that inhibits the expression of a gene that is up-regulated during Th1 polarization or that inhibits the activity of the protein it encodes. Alternatively, an agent can be administered that activates the expression of a gene that is up-regulated in Th2 cells, or which increases the activity of the protein

encoded by such a gene. A third option is to administer one or more agents that achieve both of these results.

If instead the disease is associated with an excessive Th2 response, then some treatment strategies involve administering an agent that inhibits the expression or activity of a gene that is up-regulated in Th2 cells. Alternatively, an agent is administered that activates the expression or activity of a gene that is up-regulated in Th1 cells. Still other methods involve providing an agent or agents that accomplishes both of these results.

A number of methods that are known in the art can be utilized to modulate gene expression or activity. Various agents can be used to inhibit gene expression or the activity of the corresponding protein. Examples of such agents include antisense oligonucleotides, ribozymes, triple helix structure and double-stranded RNA (dsRNA), particularly small-interfering RNAs (siRNAs). These agents are discussed in additional detail below. Alternatively, compounds that antagonize the activity of the protein encoded by the up-regulated genes can also be utilized. Examples include antibodies that specifically bind to the encoded protein. Other antagonists are small molecules.

Various options are also available for increasing gene expression or the activity of the protein encoded by a gene. One option is to administer a nucleic acid that encodes the protein whose activity one seeks to increase. This nucleic acid is operably linked to an appropriate expression control elements to facilitate its expression lymphocytes. Another option is to administer the protein itself, or an active fragment thereof. Yet another option is to administer an agonist that increases the activity of the protein.

VIII. Compounds for Inhibiting or Enhancing the Synthesis or Activity of Control Genes

A. Activity or Synthesis Inhibition

As discussed above, expression of certain genes can cause or worsen the symptoms of an immune-related disease. The increase in the expression or activity of such control genes and their products can be countered using various methodologies to inhibit their expression, synthesis or activity.

For example, antisense, ribozyme, triple helix molecules and antibodies can be utilized to ameliorate the negative effects of such control genes and gene products. Antisense RNA and DNA molecules act directly to block the translation of mRNA by hybridizing to targeted mRNA, thereby blocking protein translation. Hence, a useful target for antisense molecules is the translation initiation region.

Ribozymes are enzymatic RNA molecules that hybridize to specific sequences and then carry out a specific endonucleolytic cleavage reaction. Thus, for effective use, the ribozyme should include sequences that are complementary to the target mRNA, as well as the sequence necessary for carrying the cleavage reaction (see, *e.g.*, U.S. Pat. No. 5,093,246).

Nucleic acids utilized to promote triple helix formation to inhibit transcription are single-stranded and composed of dideoxyribonucleotides. The base composition of such polynucleotides is designed to promote triple helix formation via Hoogsteen base pairing rules and typically require significant stretches of either pyrimidines or purines on one strand of a duplex.

Double stranded RNA (dsRNA) inhibition methods can also be use to inhibit expression of one or more of the differentially expressed nucleic acids. The RNA utilized in such methods is designed such that a least a region of the dsRNA is substantially identical to a region of a differentially expressed nucleic acid (e.g., a target gene); in some instances, the region is 100% identical to the target. For use in mammals, the dsRNA is typically about 19-30 nucleotides in length (i.e., small inhibitory RNAs are utilized (siRNA)). Methods and compositions useful for performing dsRNAi and siRNA are discussed, for example, in PCT Publications WO 98/53083; WO 99/32619; WO 99/53050; WO 00/44914; WO 01/36646; WO 01/75164; WO 02/44321; and published U.S. Patent application 10/195,034, each of which is incorporated herein by reference in its entirety for all purposes.

Antibodies having binding specificity for a target gene protein that also interferes with the activity of the gene protein can also be utilized to inhibit gene protein activity. Such antibodies can be generated from full-length proteins or fragments thereof according to the methods described below.

B. Activity Enhancement

Immune-related diseases can be exacerbated by under-expression of certain control genes and/or by a reduction in activity of a control gene product, for example. Alternatively, the up-regulation of certain control gene products can produce a beneficial effect. In any of these scenarios, it is useful to increase the expression, synthesis or activity of such control genes and proteins.

These goals can be achieved, for example, by increasing the level of control gene product or the concentration of active gene product. In one approach, a control gene /

protein in the form of a pharmaceutical composition such as that described below is administered to a subject suffering from an immune-related disease. Alternatively, DNA sequences encoding control gene proteins can be administered to a patient at a concentration sufficient to treat a immune-related disease or to treat an individual at risk for such a disease. Gene therapy is yet another option and includes inserting one or more copies of a normal control gene, or a fragment thereof capable of producing a functional control protein, into lymphocytic cells using various vectors. Suitable vectors include, for example, adenovirus, adeno-associated virus and retrovirus vectors. Liposomes and other particles capable of introducing DNA into cells can also be utilized in some instances. Cells, typically autologous cells, that express a normal control gene can than be introduced or reintroduced into a patient to treat the immune-related disease.

X. Antibodies

Antibodies that are immunoreactive with polypeptides expressed from the differentially expressed genes or fragments thereof are also provided. The antibodies can be polyclonal antibodies, distinct monoclonal antibodies or pooled monoclonal antibodies with different epitopic specificities.

A. Production of Antibodies

The antibodies can be prepared using intact polypeptide or fragments containing antigenic determinants from proteins encoded by differentially expressed genes or target genes as the immunizing antigen. The polypeptide used to immunize an animal can be from natural sources, derived from translated cDNA, or prepared by chemical synthesis. In some instances the polypeptide is conjugated with a carrier protein. Commonly used carriers include keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid. The coupled peptide is then used to immunize the animal (*e.g.*, a mouse, a rat, or a rabbit). Various adjuvants can be utilized to increase the immunological response, depending on the host species and include, but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol and carrier proteins, as well as human adjuvants such as BCG (bacille Calmette-Guerin) and Corynebacterium parvum.

Monoclonal antibodies can be made from antigen-containing fragments of the protein by the hybridoma technique, for example, of Kohler and Milstein (Nature, 256:495-497, (1975); and U.S. Pat. No. 4,376,110, incorporated by reference in their

entirety). See also, Harlow & Lane, *Antibodies, A Laboratory Manual* (C.S.H.P., NY, 1988), incorporated by reference in its entirety. The antibodies can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof.

Techniques for generation of human monoclonal antibodies have also been described, including, for example, the human B-cell hybridoma technique (Kosbor *et al.*, *Immunology Today* 4:72 (1983), incorporated by reference in its entirety); for a review, *see also*, Larrick *et al.*, U.S. Pat. No. 5,001,065, (incorporated by reference in its entirety). An alternative approach is the generation of humanized antibodies by linking the complementarity-determining regions or CDR regions (*see, e.g.*, Kabat *et al.*, "Sequences of Proteins of Immunological Interest," U.S. Dept. of Health and Human Services, (1987); and Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987)) of non-human antibodies to human constant regions by recombinant DNA techniques. *See* Queen *et al.*, *Proc. Natl. Acad. Sci. USA* 86:10029-10033 (1989) and WO 90/07861 (incorporated by reference in its entirety). Alternatively, one can isolate DNA sequences that encode a human monoclonal antibody or a binding fragment thereof by screening a DNA library from human B cells according to the general protocol set forth by Huse *et al.*, *Science* 246:1275-1281 (1989) and then cloning and amplifying the sequences which encode the antibody (or binding fragment) of the desired specificity. The protocol described by Huse is rendered more efficient in combination with phage display technology. See, *e.g.*, Dower *et al.*, WO 91/17271 and McCafferty *et al.*, WO 92/01047 (each of which is incorporated by reference). Phage display technology can also be used to mutagenize CDR regions of antibodies previously shown to have affinity for the peptides of the present invention. Antibodies having improved binding affinity are selected.

Techniques developed for the production of "chimeric antibodies" by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from human antibody molecule of appropriate antigen specificity can be used. A chimeric antibody is a molecule in which different portions are derived from different species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Single chain antibodies specific for the differentially expressed gene products of the invention can be produced according to established methodologies (see, *e.g.*, U.S. Pat. No. 4,946,778; Bird, *Science* 242:423-426 (1988); Huston *et al.*, *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988); and Ward *et al.*, *Nature* 334:544-546 (1989), each of which is incorporated by reference in its entirety).

Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide.

Antibodies can be further purified, for example, by binding to and elution from a support to which the polypeptide or a peptide to which the antibodies were raised is bound. A variety of other techniques known in the art can also be used to purify polyclonal or monoclonal antibodies (*see, e.g.*, Coligan, *et al.*, Unit 9, *Current Protocols in Immunology*, Wiley Interscience, (1994), incorporated herein by reference in its entirety).

Anti-idiotype technology can also be utilized in some instances to produce monoclonal antibodies that mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region that is the "image" of the epitope bound by the first monoclonal antibody.

B. Use of Antibodies

The antibodies that are provided are useful, for example, in screening cDNA expression libraries and for identifying clones containing cDNA inserts which encode structurally-related, immunocrossreactive proteins. See, for example, Aruffo & Seed, *Proc. Natl. Acad. Sci. USA* 84:8573-8577 (1977) (incorporated by reference in its entirety). Antibodies are also useful to identify and/or purify immunocrossreactive proteins that are structurally related to native polypeptide or to fragments thereof used to generate the antibody. The antibodies can also be used to form antibody arrays to detect proteins expressed by the differentially expressed genes.

The antibodies can also be used in the detection of differentially expressed genes, such as control and fingerprint gene products. Various diagnostic assays can be utilized, including but not limited to, competitive binding assays, direct or indirect sandwich assays and immunoprecipitation assays (see, *e.g., Monoclonal Antibodies: A Manual of Techniques*, CRC Press, Inc. (1987) pp. 147-158). When utilized in diagnostic assays, the antibodies are typically labeled with a detectable moiety. The label can be any molecule capable of producing, either directly or indirectly, a detectable signal. Suitable labels include, for example, radioisotopes (*e.g.*, ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{125}I), fluorophores (*e.g.*, fluorescein and rhodamine dyes and derivatives thereof), chromophores, chemiluminescent molecules, an enzyme substrate (including the enzymes luciferase, alkaline phosphatase, beta-galactosidase and horse radish peroxidase, for example). The antibodies can also be utilized in the development of antibody arrays.

As noted above, antibodies are useful in inhibiting the expression products of the differentially expressed genes and are valuable in inhibiting the action of certain control gene products (*e.g.*, target gene products identified as causing or exacerbating tumor or cancer formation). Hence, the antibodies also find utility in a variety of therapeutic applications.

XI. Pharmaceutical Compositions

The invention provides also a pharmaceutical composition that can modulate the expression or activity of at least one gene listed in one or more of the tables (*e.g.*, Table 2 or Table 6) for use in prophylaxis or treatment of asthma or other immune-mediated disease. In a preferred embodiment said pharmaceutical composition comprises an antibody binding to at least one gene product of the genes listed in Table 2 or Table 6 as an active ingredient.

A. Composition

The pharmaceutical compositions used for treatment of cancers and tumors comprise an active ingredient such as the inhibitory or activity-enhancing compounds such as described herein and, optionally, various other components.

Thus, for example, the compositions can also include, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers of diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, buffered water, physiological saline, PBS, Ringer's solution, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation can include other carriers, adjuvants, or non-toxic, nontherapeutic, nonimmunogenic stabilizers, excipients and the like. The compositions can also include additional substances to approximate physiological conditions, such as pH adjusting and buffering agents, toxicity adjusting agents, wetting agents, detergents and the like.

The composition can also include any of a variety of stabilizing agents, such as an antioxidant for example. When the pharmaceutical composition includes a polypeptide, the polypeptide can be complexed with various well known compounds that enhance the *in vivo* stability of the polypeptide, or otherwise enhance its pharmacological properties (*e.g.*, increase the half-life of the polypeptide, reduce its toxicity, enhance solubility or uptake). Examples of such modifications or complexing agents include the production of sulfate,

gluconate, citrate, phosphate and the like. The polypeptides of the composition can also be complexed with molecules that enhance their *in vivo* attributes. Such molecules include, for example, carbohydrates, polyamines, amino acids, other peptides, ions (*e.g.*, sodium, potassium, calcium, magnesium, manganese), and lipids.

Further guidance regarding formulations that are suitable for various types of administration can be found in *Remington's Pharmaceutical Sciences*, Mace Publishing Company, Philadelphia, PA, 17th ed. (1985). For a brief review of methods for drug delivery, *see*, Langer, *Science* 249:1527-1533 (1990).

B. Dosage

The pharmaceutical compositions can be administered for prophylactic and/or therapeutic treatments. The active ingredient in the pharmaceutical compositions typically is present in a therapeutic amount, which is an amount sufficient to slow or reverse tumor formation, to eliminate the tumor, or to remedy symptoms associated with the tumor or cancer. Toxicity and therapeutic efficacy of the active ingredient can be determined according to standard pharmaceutical procedures in cell cultures and/or experimental animals, including, for example, determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds that exhibit large therapeutic indices are preferred.

The data obtained from cell culture and/or animal studies can be used in formulating a range of dosages for humans. The dosage of the active ingredient typically lines within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized.

In prophylactic applications, compositions containing the compounds that are provided are administered to a patient susceptible to or otherwise at risk of tumor formation. Such an amount is defined to be a "prophylactically effective" amount or dose. In this use, the precise amount depends on the patient's state of health and weight. Typically, the dose ranges from about 1 to 500 mg of purified protein per kilogram of body weight, with dosages of from about 5 to 100 mg per kilogram being more commonly utilized.

C. Administration

The active ingredient, alone or in combination with other suitable components, can be made into aerosol formulations (*i.e.*, they can be "nebulized") to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen.

Suitable formulations for rectal administration include, for example, suppositories, which consist of the packaged active ingredient with a suppository base. Suitable suppository bases include natural or synthetic triglycerides or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules, which consist of a combination of the packaged nucleic acid with a base, including, for example, liquid triglycerides, polyethylene glycols, and paraffin hydrocarbons.

Formulations suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions can be administered, for example, by intravenous infusion, orally, topically, intraperitoneally, intravesically or intrathecally. Formulations for injection can be presented in unit dosage form, *e.g.*, in ampules or in multidose containers, with an added preservative. The compositions are formulated as sterile, substantially isotonic and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration.

XII. Devices for Detecting Differentially Expressed Nucleic Acids

A. Customized Probe Arrays

1. Probes for Differentially Expressed Genes

The differentially expressed genes that are provided can be utilized to prepare custom probe arrays for use in screening and diagnostic applications. In general, such arrays include probes such as those described above in the section on differentially expressed nucleic acids, and thus include probes complementary to full-length differentially expressed nucleic acids (*e.g.*, cDNA arrays) and shorter probes that are typically 10-30 nucleotides long (*e.g.*, synthesized arrays). Typically, the arrays include probes capable of detecting a plurality of the differentially expressed genes of the

invention. For example, such arrays generally include probes for detecting at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 differentially expressed nucleic acids. For more complete analysis, the arrays can include probes for detecting at least 12, 14, 16, 18 or 20 differentially expressed nucleic acids. In still other instances, the arrays include probes for detecting at least 25, 30, 35, 40, 45 or all the differentially expressed nucleic acids that are identified herein.

2. Control Probes

(a) Normalization Controls

Normalization control probes are typically perfectly complementary to one or more labeled reference polynucleotides that are added to the nucleic acid sample. The signals obtained from the normalization controls after hybridization provide a control for variations in hybridization conditions, label intensity, reading and analyzing efficiency and other factors that can cause the signal of a perfect hybridization to vary between arrays. Signals (*e.g.*, fluorescence intensity) read from all other probes in the array can be divided by the signal (*e.g.*, fluorescence intensity) from the control probes thereby normalizing the measurements.

Virtually any probe can serve as a normalization control. However, hybridization efficiency can vary with base composition and probe length. Normalization probes can be selected to reflect the average length of the other probes present in the array, however, they can also be selected to cover a range of lengths. The normalization control(s) can also be selected to reflect the (average) base composition of the other probes in the array. Normalization probes can be localized at any position in the array or at multiple positions throughout the array to control for spatial variation in hybridization efficiently.

(b) Mismatch Controls

Mismatch control probes can also be provided; such probes function as expression level controls or for normalization controls. Mismatch control probes are typically employed in customized arrays containing probes matched to known mRNA species. For example, certain arrays contain a mismatch probe corresponding to each match probe. The mismatch probe is the same as its corresponding match probe except for at least one position of mismatch. A mismatched base is a base selected so that it is not complementary to the corresponding base in the target sequence to which the probe can otherwise specifically hybridize. One or more mismatches are selected such that under appropriate hybridization conditions (*e.g.* stringent conditions) the test or control probe can be expected to hybridize with its target sequence, but the mismatch probe cannot hybridize

(or can hybridize to a significantly lesser extent). Mismatch probes can contain a central mismatch. Thus, for example, where a probe is a 20 mer, a corresponding mismatch probe can have the identical sequence except for a single base mismatch (*e.g.*, substituting a G, a C or a T for an A) at any of positions 6 through 14 (the central mismatch).

(c) Sample Preparation, Amplification, and Quantitation Controls

Arrays can also include sample preparation/amplification control probes. Such probes can be complementary to subsequences of control genes selected because they do not normally occur in the nucleic acids of the particular biological sample being assayed. Suitable sample preparation/amplification control probes can include, for example, probes to bacterial genes (*e.g.*, Bio B) where the sample in question is a biological sample from a eukaryote.

The RNA sample can then be spiked with a known amount of the nucleic acid to which the sample preparation/amplification control probe is complementary before processing. Quantification of the hybridization of the sample preparation/amplification control probe provides a measure of alteration in the abundance of the nucleic acids caused by processing steps. Quantitation controls are similar. Typically, such controls involve combining a control nucleic acid with the sample nucleic acid(s) in a known amount prior to hybridization. They are useful to provide a quantitative reference and permit determination of a standard curve for quantifying hybridization amounts (concentrations).

3. Array Synthesis

Nucleic acid arrays for use in the present invention can be prepared in two general ways. One approach involves binding DNA from genomic or cDNA libraries to some type of solid support, such as glass for example. (*See, e.g.*, Meier-Ewart, *et al.*, *Nature* 361:375-376 (1993); Nguyen, C. *et al.*, *Genomics* 29:207-216 (1995); Zhao, N. *et al.*, *Gene*, 158:207-213 (1995); Takahashi, N., *et al.*, *Gene* 164:219-227 (1995); Schena, *et al.*, *Science* 270:467-470 (1995); Southern *et al.*, *Nature Genetics Supplement* 21:5-9 (1999); and Cheung, *et al.*, *Nature Genetics Supplement* 21:15-19 (1999), each of which is incorporated herein in its entirety for all purposes.)

The second general approach involves the synthesis of nucleic acid probes. One method involves synthesis of the probes according to standard automated techniques and then post-synthetic attachment of the probes to a support. See for example, Beaucage, *Tetrahedron Lett.*, 22:1859-1862 (1981) and Needham-VanDevanter, *et al.*, *Nucleic Acids*

Res., 12:6159-6168 (1984), each of which is incorporated herein by reference in its entirety. A second broad category is the so-called "spatially directed" polynucleotide synthesis approach. Methods falling within this category further include, by way of illustration and not limitation, light-directed polynucleotide synthesis, microlithography, application by ink jet, microchannel deposition to specific locations and sequestration by physical barriers.

Light-directed combinatorial methods for preparing nucleic acid probes are described in U.S. Pat. Nos. 5,143,854 and 5,424,186 and 5,744,305; PCT patent publication Nos. WO 90/15070 and 92/10092; EP 476,014; Fodor *et al.*, *Science* 251:767-777 (1991); Fodor, et al., *Nature* 364:555-556 (1993); and Lipshutz, *et al.*, *Nature Genetics Supplement* 21:20-24 (1999), each of which is incorporated herein by reference in its entirety. These methods entail the use of light to direct the synthesis of polynucleotide probes in high-density, miniaturized arrays. Algorithms for the design of masks to reduce the number of synthesis cycles are described by Hubbel *et al.*, U.S. 5,571,639 and U.S. 5,593,839, and by, Fodor *et al.*, *Science* 251:767-777 (1991), each of which is incorporated herein by reference in its entirety.

Other combinatorial methods that can be used to prepare arrays for use in the current invention include spotting reagents on the support using ink jet printers. See Pease et al., EP 728, 520, and Blanchard, *et al. Biosensors and Bioelectronics* II: 687-690 (1996), which are incorporated herein by reference in their entirety. Arrays can also be synthesized utilizing combinatorial chemistry by utilizing mechanically constrained flowpaths or microchannels to deliver monomers to cells of a support. See Winkler *et al.*, EP 624,059; WO 93/09668; and U.S. Pat. No. 5,885,837, each of which is incorporated herein by reference in its entirety.

4. Array Supports

Supports can be made of any of a number of materials that are capable of supporting a plurality of probes and compatible with the stringency wash solutions. Examples of suitable materials include, for example, glass, silica, plastic, nylon or nitrocellulose. Supports are generally are rigid and have a planar surface. Supports typically have from 1-10,000,000 discrete spatially addressable regions, or cells. Supports having 10-1,000,000 or 100-100,000 or 1000-100,000 regions are common. The density of cells is typically at least 1000, 10,000, 100,000 or 1,000,000 regions within a square centimeter. Each cell includes at least one probe; more frequently, the various cells

include multiple probes. In general each cell contains a single type of probe, at least to the degree of purity obtainable by synthesis methods, although in other instances some or all of the cells include different types of probes. Further description of array design is set forth in WO 95/11995, EP 717,113 and WO 97/29212, which are incorporated by reference in their entirety.

XIII. Kits

Kits containing components necessary to conduct the screening and diagnostic methods of the invention are also provided. Some kits typically include a plurality of probes that hybridize under stringent conditions to the different differentially expressed nucleic acids that are provided. Other kits include a plurality of different primer pairs, each pair selected to effectively prime the amplification of a different differentially expressed nucleic acid. In the case when the kit includes probes for use in quantitative RT-PCR, the probes can be labeled with the requisite donor and acceptor dyes, or these can be included in the kit as separate components for use in preparing labeled probes.

The kits can also include enzymes for conducting amplification reactions such as various polymerases (*e.g.*, RT and Taq), as well as deoxynucleotides and buffers. Cells capable of expressing one or more of the differentially expressed nucleic acids of the invention can also be included in certain kits.

Typically, the different components of the kit are stored in separate containers. Instructions for use of the components to conduct an analysis are also generally included.

The following examples are offered to illustrate certain aspects of the methods and devices that are provided; it should be understood that these examples are not to be construed to limit the claimed invention.

EXAMPLES

A. Materials and Methods

Induction of Th1 and Th2 in vitro polarization

The mononuclear cells were isolated from the human cord blood of healthy neonates using Ficol Isolation Paque (Amersham Pharmacia Biotech, Uppsala, Sweden). The CD4+ cells were further purified using magnetic beads (Dynal, Oslo, Norway). The primary activation was performed using plate-bound αCD3 (500-1000 ng/µl for coating) and 500 ng/µl soluble αCD28 (Immunotech, Marseille, France). The cells were cultured in

the density of 0.5 - 2 x 10^6 cells/ml in Yssel's medium (Irvine Scientific, Santa Ana, CA) containing 1% AB-serum (Red Cross, Helsinki, Finland). The polarization of the cells was performed in either Th1 medium containing 2.5 ng/ml of IL-12 (R&D Systems, Minneapolis, MN) or in Th2 medium containing 10 ng/ml of IL-4 (R&D Systems) in the presence and absence of 3 ng/ml TGFβ (R&D Systems). Part of the cells was cultured in "neutral conditions" without any polarizing cytokines. Samples were collected at the time points of 0h, 2 h, 6h and 48 h.

Real-Time quantitative RT-PCR

Real-time quantitative RT-PCR was performed to control the quality of the cell samples with different treatments. The gene expression levels were measured for the *IFNγ, GATA-3, T-bet* and housekeeping gene *Elongation factor 1 alpha* (*EF1α),* using TaqMan ABI Prism 7700 (TaqMan, ABI Prism 7700, Applied Biosystems, Foster City, CA) as described before (18). *EF1α* was used as a reference transcript. The expression of this housekeeping gene remains stable during the differentiation of Th1 and Th2 cells (19). Primers and probes (Table 4) used for the quantification of gene expression (MedProbe, Norway) were designed using Primer Express software (Applied Biosystems).

Oligonucleotide array studies

The total RNA of the samples was isolated using the Trizol method (Invitrogen Co., Carlsbad, CA) and was further purified with Qiagen's RNAeasy minikit (Qiagen, Valencia, CA). 4-5 μg of totRNA was used as starting material for the sample preparation. The sample preparation was performed according to the instructions and recommendations provided by the probe array manufacturer (Affymetrix). The samples were hybridized to HG-U95Av2 arrays containing probes for approximately 10,000 genes. The data was analyzed on three consecutive levels. At the detection level, each probe was assigned a call of present, absent, marginal, or no call. The comparison level analysis of the cells cultured defines a gene as upregulated if the signal log ratio between the reference and the target samples is larger than one (2-fold increase) and the target sample is present. Similarly, a gene is defined as downregulated if the signal log ratio is less than minus one (2-fold decrease) and the reference sample is present. At the third level of data analysis, genes that presented a consistent change in two separate experiments were considered as differentially expressed. The gene transcript levels were determined from data images with the algorithms in the GeneChip Microarray Suite™ software (Affymetrix MAS5), and

the subsequent gene filtering and data analysis was done with Microsoft Excel 2002, Microsoft Access 2000 softwares.

Validation of the oligonucleotide array results

For validation of the oligonucleotide array results with Real-Time RT-PCR, additional Th1 and Th2 primary cultures were generated as previously described (18). Briefly, the priming was performed in the presence of 100 ng/ml PHA (Murex Diagnostics, Chatillon, France) and irradiated CD32-B7 transfected fibroblasts (20). The feeder cells were added in the final density of 1 x 10^6 cells/ml. Th1 cultures were supplemented with 2.5 ng/ml of IL-12 (R&D Systems, Minneapolis, MN). Th2 cultures were supplemented with 10 µg/ml of anti-IL-12 (R&D Systems) and 10 ng/ml of IL-4 (R&D Systems). After 48 hours of priming, 40 U/ml of IL-2 (R&D Systems) was added into the cultures to enhance the proliferation of the lymphocytes. Part of the cells were cultured without any polarizing cytokines in the presence of IL-2 alone. The cultures were generated from four individuals, and during polarization samples were collected at the time points 0 h, 6 h, 24 h, 48 h and 7 d.

Mice, reagents and cell culture

Stat6 deficient mice and control wild-type Balb/cJ mice were from Jackson Laboratory (Bar Harbor, Maine). Splenic mononuclear cells were isolated with Ficoll-Paque PLUS (Amersham Pharmacia Biotech, Uppsala, Sweden). $CD4^+$ T lymphocytes were further purified by magnetic CD4 MicroBeads (Miltenyi Biotech, Bergisch Gladbach, Germany). The cells were activated with plate-bound anti-mouse CD3 (clone 500A2, 1.26µg/ml, Pharmingen, San Diego, CA) and soluble anti-mouse CD28 (500ng/ml; Pharmingen, San Diego, CA) and were cultured in IDME media containing 10% FCS, nonessential amino acids and 2-mercaptoethanol (all from Gibco BRL, Life Technologies, Paisley, Scotland). RmIL-4 (10ng/ml, Pharmingen, San Diego, CA) and anti-IL-12, anti-IFN-γ (both are 10µg/ml, Pharmingen, San Diego, CA) were added for Th2 development. $CD4^+$ T cells pooled from different mice were cultured as Th0 (with activation and anti-IL-4, anti-IL-12 and anti-IFN-γ) or Th2 differentiation. Cells were harvested at 0h, 2h, 6h, 24h and 48h.

Oligonucleotide microarray analysis

Total RNA was extracted by Trizol (Gibco BRL, Life Technologies, Paisley, Scotland) and further purified with RNeasy Kit (Qiagen, Valencia, CA). cDNA was

synthesized by Superscript II kit (Gibco BRL, Life Technologies, Paisley, Scotland) using T7-$(dT)_{24}$ as primer. Biotin-labelled cRNA was prepared by *in vitro* transcription reaction using BioArray HighYield RNA Transcript Labelling Kit (Enzo Diagnostics, Inc. Farmingdale, NY) based on manufacture's protocol. The cRNA was purified, fragmented and hybridized to Affymetrix MG-U74A Genechips™. Arrays were stained and scanned according to Affymetrix (Santa Clara, CA) protocols.

Affymetrix Microarray Suit™ (version 5.0) software was used for the data analysis. In this program, Signal Log Ratio is used to describe the change between a target and reference array. The change is expressed as $\log_2$ ratio. Therefore, $\log_2$ ratio (Signal Log Ratio) of 1 equal to a two-fold change. In this study, the probe sets were excluded if: 1), the detection for both target and reference is Absent, 2) in comparison analysis, if the Change call gives NC (No Change) and 3), the signal log ratio between target and reference is between –1 and 1. Since there are five independent cell cultures to study IL4 inducible genes at 48h, *t-test* was also used to compare the signals between target (Activation + IL4) and reference (Activation) groups. And then 0.05 was set as the cutoff for *p-value.*

Real time quantitative pcr (TaqMan®) detection

The principle of TaqMan® detection has been described previously. Primers and probes for TaqMan® detection were designed by Primer Express™ software (Applied Biosystems, Foster City, CA) and made by MedProbe (Oslo, Norway). The sequences for the primers and probes are listed in Table 5. Samples from three independent cultures were measured in duplicate in two separate runs. The standard deviation from these values must be less than the 0.5. C_T value, which means the number of PCR cycles required for the detection of fluorescence signal to exceed a fixed threshold. The relative expressions of target mRNA were normalized against EF1α:

$$\Delta C_T = C_T (EF1\alpha) - C_T (target)$$

$$\Delta\Delta C_T = \Delta C_{T1} - \Delta C_{T2}$$

Where ΔC_{T2} represents ΔC_T from not treated (time point 0h) Stat6 +/+ and Stat6 -/- $CD4^+$ T cells and ΔC_{T1} represents ΔC_T from other time points. With this calculation, basically $\Delta\Delta C_T$ equals to $\log_2$ ratio, therefore $\Delta\Delta C_T$ 1 = 2 fold changes. The experiments were performed using an ABI PRISM 7700 Sequence Detection System (Applied Biosystems).

Immunofluorescence staining and facs analysis

$CD4^+$ T cells from Stat6 +/+ and Stat6 -/- mice were activated and cultured for Th2 priming. At 48h, samples were collected and stained. Briefly, cells were washed with PBS and then stained with FITC-conjugated rat anti-mouse Ly-6A/E monoclonal antibody (BD Biosystem, San Diego, CA) or isotype control antibody at 4°C for 20 min. Cells were washed twice and then analysed by FACScan with CellQuest software. At least 10000 cells were analysed for each sample.

B. Results

In order to elucidate the genes involved in the early polarization and to explore the inhibitory mechanism of TGFβ on the differentiation of Th1 and Th2 cells, we studied the gene expression profiles of the CD4+ cells induced to Th1 and Th2 directions in the absence and presence of TGFβ. First, the differential expression of known Th1 and Th2 marker genes *IFNγ, T-bet* and *GATA-3* was studied in the 48-hour samples to ensure that the cells had been induced to differentiate to Th1 and Th2 directions (FIG. 1) (13, 16). After that the samples were hybridized on HG-U95Av2 arrays representing probes for approximately 10,000 mainly known human genes. According to oligonucleotide array results, activation via CD3/CD28 alone induced expression of 437 probe sets and repressed 361 probesets at 2-hour time point (data not shown). After 6 hours expression of 832 probe sets was upregulated, whereas 856 probes sets were downregulated in response to CD3/CD28-activation (data not shown). After 48 hours, 582 probe sets become induced and 533 probes sets were repressed by CD3/CD28-activation compared to the Thp cells (FIG. 2A). In the figure only the genes showing 16-fold change in comparison between activated and Thp cells are presented. In addition to genes regulated by activation, numerous targets with various functions were identified as being regulated by the cytokines IL-12, IL-4 and TGFβ (see Tables 1 and 2).

Targets of IL-12 and IL-4

The effects of IL-12 were modest during the immediate phase (2 and 6 hours) of polarization as over 2-fold changes were seen in expression of only 3 genes (Table 1). All of these were classified to be "not changed" by the MAS5 algorithm and thus might be not reliable changes. After 48-hours the effects of IL-12 become clear as 23 genes become regulated by IL-12 (Fig. 2B). Altogether 40 genes were detected to be differentially expressed by the cells induced to differentiate to Th1 or Th2 directions for 48 hours (Table

1 and FIG. 2F). Previously reported "marker genes" *IFNG, IL-2, IL18RAP, CTLA1/GZMB, G0S2, ANXA3, P2RX5, LIF* and *BLR1* were preferentially expressed by the cells induced to polarize to the Th1 direction (21-23) and *MAF/C-MAF, GATA-3, EBI2, IL10RA, Cox-2, NTRK1, CXCR4, E4BP4/ NFIL3* and *IL-13* were confirmed to be preferentially expressed by the cells induced to the Th2 direction (15, 16, 21, 22, 24-26). Moreover, suppressor of cytokine signaling 1 *(TIP3/SOCS-1)* was significantly more expressed by the cells polarized to the Th2 direction compared to those induced to the Th1 direction.

The effects of IL-4 were clear already after 2 hours of Th2 polarization when 34 genes become regulated by IL-4 compared to the CD3/CD28-activation (Table 1). After 6 hours, 38 genes were detected to be regulated by IL-4. After 48 hours IL-4 regulated expression of 41 genes (Table I and FIG. 2C). These genes included both known and unknown IL-12 and IL-4 regulated genes. To our knowledge, regulation of the genes *PACE, MRF-1, FLOT1, MTMR1, GOSR2, AF055029, AL050166, AF070528, SCYC2* by IL-12 and genes *KIAA1013, ID2, KIAA0750, STK17B, HMGCS1, AL049940, AUH, BCL2A1* and *HIC* by IL-4 has not been previously described.

Regulation of gene expression by TGFβ

TGFβ regulated expression of numerous genes after 2 or 6 hours in Th1 or Th2 in polarizing conditions (Table I). However, only few of these genes (ID3, CCL20, RTP801, LAMA3, R32184_3) were also regulated by IL-4 or IL-12. After 48 hours the antagonizing effects of TGFβ on the genes regulated by IL-12 or IL-4 become more evident. The presence of TGFβ in Th1 conditions induced the expression of 16 genes and repressed expression of 6 genes (see Table I and FIG. 2D). These TGFβ target genes included specific targets of TGFβ, but also genes regulated by IL-12 or IL-4. TGFβ antagonized the effects of IL-12 by upregulating expression of TNFRSF9 and by repressing expression of GZMB/CTLA-1, a gene induced by both IL-12 and IL-4. Interestingly, in Th1 conditions TGFβ also downregulated the expression of IL-4-inducible genes NFIL3/E4BP4 and SATB1 and induced expression of VIM, which was preferentially expressed by the cells cultured in Th2 conditions when compared to those cultured in Th1 conditions.

In Th2 conditions TGFβ induced expression of 16 genes and repressed 15 genes (see Table I and FIG. 2E). Again TGFβ regulated a specific set of its own targets, but importantly it antagonized the effects of IL-4 by repressing a set of IL-4-inducible genes

(ID2, Cox-2, PLA2G4A, BCL2A1, NFIL3) and expression of *GNAI1*, which was preferentially expressed in the cells cultured in Th2 conditions when compared to Th1 conditions.

Validation of a set of genes using Real-Time RT-PCR

Oligonucleotide array results were obtained from two independent experiments. Four of the genes (*SATB1, DUSP6, E4BP4* and *TIP3)* identified to be differentially expressed by the cells cultured in Th1 and Th2 conditions were selected for TaqMan RT-PCR analysis to further validate the results obtained with oligonucleotide arrays and to follow the expression kinetics of this set of interesting genes in Th1 and Th2 conditions during the one week of polarization from Thp cells. The results obtained with Real-Time RT-PCR were concordant with the oligonucleotide array data (FIG. 3). Importantly, *NFIL3/E4BP4, TIP3/SOCS-1* and *DUSP6* were identified to be differentially expressed in the cells cultured in Th1 and Th2 conditions already after six hours of polarization and the differences were maintained for at least the two first days of polarization. Also *SATB1* was differentially expressed after six hours in three of four individuals studied, and in all individuals after one and two days of polarization. *DUSP6* is expressed as two alternative splicing variants, and thus expression of both forms was quantitated. According to the results, only the long form is expressed by the cells studied. Also the expression of gene *GADD45β* was verified with RT-PCR based on the data analysis with the previous version of the Affymetrix analysis program, MAS4™, which found the gene to be preferentially expressed by the cells cultured in Th1 conditions compared to Th2 conditions as described before (27, 28). However, after reanalysis of the data with the updated MAS5 program, *GADD45β* was excluded from the results, and therefore, not presented in the FIG. 2. The RT-PCR analysis confirmed the differences in the expression of *GADD45β* in the cells cultured in Th1 or Th2 conditions.

IL4 inducible genes in murine CD4$^+$ T lymphocytes

To identify IL4 inducible genes in murine CD4$^+$ T lymphocytes, CD4$^+$ T cells were isolated from Stat6 $^{+/+}$ mice (Balb/cJ mice) and were activated with plate-bound anti-CD3 and soluble anti-CD28. IL4, anti-IL12 and anti-IFNγ were added for Th2 development. By comparing microarray data from Th2 (IL4+activation) vs. Th0 (activation alone) at 48h, IL4 inducible genes were identified. From 5 independent cell cultures, 117 probe sets (116 known genes) were regulated by IL4, 26 of 117 were up-

regulated and 91 of them were down-regulated (Table 3). The differentially expressed genes were divided to 6 groups: immune response and related, transcription regulation and DNA binding, enzymes and inhibitors, apoptosis and cell proliferation, cell surface and structure proteins and miscellaneous.

Stat6 regulated murine genes

As T cells from Stat6 $^{-/-}$ mice have the deficiency for Th2 development, we isolated both Stat6 $^{-/-}$ and Stat6 $^{+/+}$ $CD4^+$ T cells, activated and cultured them for Th2 polarization. At 48h, cells were harvested; RNA was isolated and subjected to DNA microarray analysis. Stat6 target genes were identified by comparing DNA microarray data obtained from Stat6 $^{-/-}$ Th2 (IL4+activation) cells to data obtained from Stat6 $^{+/+}$ Th2 (IL4+activation) cells. 37 probe sets (36 known genes) were differentially expressed. 17 (including Stat6) of 37 probe sets were down-regulated and 20 probe sets were downregulated in Stat6 deficient cells as compared to the wild type cells (Table 3). Within these genes, 20 are induced by IL4, including the known Stat6 target genes, IL4 and Gata3.. Hipk2 and Nfil3 have been shown to be Stat6 target genes also in B cells (29). Stat6 is able to compete with Stat1 for its binding to the IFNγ promoter and inhibit its expression (30, 31, 32). Therefore, it makes sense that a number of IFN regulated genes, such as Ifi205, Ifi203, Isg15 and Isg20, were up-regulated in Stat6 $^{-/-}$ $CD4^+$ T cells. Expression of certain genes known to be expressed mainly in Th1 cells or induced by IFNγ, including Il18r1, CCR5, Gzmb, Ly6a, and Txk, was inhibited by Stat6.

20 of 37 known Stat6 target genes are induced by IL4, whereas 20 of 117 IL4 inducible genes are regulated via Stat6 (Table 3). Because there are other signal pathways involved in IL4 signalling (33), it is evident that not all the IL4 inducible genes are regulated by Stat6. Besides the 20 IL4 inducible and Stat6 regulatory genes, there were 17 Stat6 regulated genes that are not induced by IL4.

Kinetic study of IL4 inducible and Stat6 regulated genes in mice

In order to elucidate the molecular mechanisms for T cell differentiation at very early stage and also to follow the early changes for those IL4 and Stat6 regulated genes identified at 48h, the kinetics of gene expression profiles at early T cell polarization were studied by using oligonucleotide arrays (Affymetrix). At early time points(2h and 6h), a group of novel IL4 inducible genes were identified, including Bcl2, Atf3, Pole2, Cish and Crabp2. Il4ra and Pros1, twoknown IL4 inducible genes, were induced by IL4 already at early time points. Fig1 was induced by IL4 at 2h and remained upregulated at 24h.

Interestingly, there were more genes induced by IL4 at early time points; on the contrary, there were more genes suppressed by IL4 at 48h. Most Th1 marker genes or interferon inducible genes, such as Il18r1, Ifi203, Ifi204, Ifi205, Ifng, Ifit1, became differentially expressed at 24h or 48h.**[0002]** The kinetic study, revealed a group of early Stat6 regulated genes, such as Pole2, Ppp3cc, Fig1, Pros1, Casp6, Il4ra and IL4. All of these genes were also IL4 inducible genes. At 24h and 48h, Myo6, Cmkbr8 (CCR8), Crabp2, Hipk2, Atf3, Nfil3, Zfp118 were identified as Stat6 regulated genes

The novel IL4 inducible or Stat6 target genes in murine cells identified in this study are summarized in Table 6.

Taqman and FACS analysis confirms the microarray results

To verify the findings from microarray data, selected genes were further studied by real-time quantitative RT-PCR. Based on microarray results, Hipk2, Nfil3, Zfp118 and Atf3 are induced by IL4 and are regulated by Stat6. Expression of Ifi203 is inhibited by IL4 via Stat6. Expression of these genes was measured using real-time PCR during the first two days of $CD4^+$ T cell differentiation using samples from three independent cell cultures. In Stat6 +/+ $CD4^+$ T cells the expression of Hipk2 was induced by IL4 quickly (2h) and the difference in Hipk2 expression between Th1 and Th2 cells further increased at 24h and 48h. Hipk2 expression was lower in Stat6-deficient cells compared to wild type cells at all the time points. Similar results were obtained for Nfil3 and Zfp118. Atf3 was induced by IL4 already at 2h. In Stat6 -/- $CD4^+$ T cells, the expression level of Atf3 in Th2 condition was lower than in Stat6 +/+ T cells. In contrast, the expression of Ifi203 was inhibited by IL4. When Stat6 is deficient, especially at 48h, its expression level was increased and the biggest difference was seen quite late (48h). We consider that Ifi203 could be a secondary target of Stat6. Together, these results show that these genes were induced or repressed by IL4 through Stat6.

The Ly-6 family of cell surface molecules has previously been shown to participate in T cell activation. The expression of Ly-6A/E is upregulated on normal murine T and B cells by IFN-gamma. The protein expression of Ly6a at 48h polarized Th2 cells was measured by FACScan using FITC-conjugated rat anti-mouse Ly-6A/E monoclonal antibody. As expected based on microarray results, at 48h the protein expression of Ly6a was upregulated (90%) in Stat6 -/- cells cultured in Th2 conditions compared to wild type cells (45%) cultured equally.

C. Discussion

Our study demonstrates that triggering of the T cell receptor leads to regulation of more than 1000 genes with various functions. Compared to that number, the group of target genes regulated by cytokines mediating the differentiation process is specific and limited to only less than 100 genes, at least at this stage of differentiation.

Comparison of the cells cultured in Th1 or Th2 conditions to each other or to CD3/CD28-activated cells revealed changes in the expression of 124 genes. Of these genes approximately 77 have not been previously associated with Th1 and Th2 polarization. Differential regulation of genes *DUSP6, E4BP4/NFIL3, SATB1* and *TIP3/SOCS-1* was further confirmed using Real-Time RT-PCR. Preferential induction of these genes by IL-4 already after 2 hours of Th2 polarization positions these genes as important candidates as upstream regulators of the early differentiation process. The roles of these genes in Th1 and Th2 differentiation are currently unknown. DUSP6 is a phosphatase, which inhibits activity of ERK2 (35). E4BP4/NFIL3 is an inducer or repressor of transcription, which can activate *IL-3* expressions, and a binding site for this factor is also present in the promoter area of *IFNγ*. In pro-B lymphocytes E4BP4/NFIL3 has been found to participate in preventing apoptosis in response to IL-3 through ras-mediated signaling, which involves activation of both PI3K and raf/MAPK pathways (36, 37). SATB1 is a DNA binding protein, which is known to be involved in the development of thymic T cells (38). Its cleavage by Caspase-6 and resulting dissociation from chromatin is involved in nuclear degradation occurring during early apoptosis of T cells (39). TIP3/SOCS-1 induced by cytokine/STAT pathway is an inhibitor of cytokine signaling. Previous reports have provided controversial information concerning the role of TIP3/SOCS-1 in Th1 and Th2 cells; on one hand the protein has been shown to be preferentially expressed by the Th1 cells in mouse (40). On the other hand, it has been reported that TIP3/SOCS-1 is induced by IL-6 in human, which promotes the Th2 and inhibits the Th1 commitment (40, 41). DUSP6, SATB1, E4BP4/NFIL3 and TIP3/SOCS-1 are likely to be among those upstream factors that respond first to the polarizing signals and thus are involved in determining the fate of Thp cells during the early stages of polarization.

It has been previously shown that TGFβ inhibits the differentiation of Th1 and Th2 subtypes (42, 43). Studies with mice have shown that the inhibition of Th1 and Th2 differentiation by TGFβ occurs through suppressing *T-bet* and *GATA-3* expression, respectively (44-46). In our study with human cells these two genes were not among the numerous primary genes regulated by TGFβ indicating that the mechanism of TGFβ

regulation on Th1 and Th2 responses is probably more complex than has been previously thought. The effects of TGFβ can also vary depending on the stage of cell differentiation, cytokine environment and concentration of TGFβ used in the cultures (47).

Enhanced Th2 response is known to contribute to the phenotype and symptoms of asthma (1-2). On the other hand, recent studies with mouse models have demonstrated that TGFβ1 can suppress the airway hyper-responsiveness and airway inflammation associated with asthma (48, 49). However, the molecular mechanism of this suppression is not known. One hypothesis could be that TGFβ is able to inhibit pathogenesis of asthma through its ability to suppress Th2 responses. If this hypothesis is correct, the genes which are regulated by TGFβ to suppress the Th2 development are particularly interesting. Our results demonstrate that TGFβ regulates a set of the same target genes as IL-12 and IL-4 *(ID3, CCL20, RTP801, LAMA3, R32184_3, TNFSF9, E4BP4, CTLA1/GZMB, ID2, Cox-2, GNAI1, PLA2G4A, BCL2A1).* Interestingly, most of these genes coregulated by IL-12/IL-4 and TGFβ are known to participate in the signaling events involved in apoptosis or survival. The IL-12 or IL-4 antagonizing influence of TGFβ on the expression of these genes could partly explain the inhibitory effect of TGFβ on differentiation. If regulation of the expression of these genes can regulate the differentiation process, they might be critical factors for the polarization.

In addition to genes regulated by IL-12 and IL-4, TGFβ also regulated many other interesting targets such as *DUSP4, IL-9, IL1RN* and *LGAL3*. *DUSP4*, which is known to inhibit ERK1/2, JNK1/2 and c-jun activity, was upregulated by TGFβ in Th1 conditions (50-51). JNK1 and JNK2 have been associated with Th1 and Th2 differentiation (52-53). Studies with knockout mice have demonstrated that in the absence of JNK1, the differentiation of Th1 and Th2 subsets is impaired and the cells preferentially polarize to Th2 subtypes. JNK2 seemed to be required for proper Th1 differentiation. Inhibition of JNK2 activity through DUSP4, could provide one possible mechanism responsible for inhibiting Th1 differentiation by TGFβ. Interestingly, in Th2 conditions TGFβ induced expression of certain genes that have been previously connected to asthma and other inflammatory diseases. These genes included such as *IL1RN, IL-9* and *LGAL3*. IL1RN is known to inhibit the IL-1 signaling by binding to IL1R, which according to our results is upregulated by IL-4. Polymorphisms in the *IL1RN* gene have been associated with various inflammatory diseases, such as asthma and diabetes (54, 55). IL-9 is a Th2-type cytokine and a candidate gene for asthma (56). It is involved in induction of many symptoms

associated with asthma and has been considered as a potential target for asthma therapy (57). LGAL3 is involved in downregulation of Th2 cytokine IL-5 and it has been used to repress the symptoms of asthma in rat models (58, 59). Taken together, in addition to antagonizing the effects of IL-12 and IL-4, in Th2 conditions TGFβ regulates expression of various genes involved in inflammatory diseases. These genes are likely to play an important role in maintaining the balance between Th1 and Th2 responses.

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It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent or patent application were specifically and individually indicated to be so incorporated by reference.

Table 1

Target genes of IL-12 in human CD4+ T cells (Th1-induced vs activated)

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
2h	32417_at	desmocollin 3	DSC3	D17427	2.8*
	37194_at	GATA binding protein 2	GATA2	M68891	2.1*
	39718_r_at	mitochondrial ribosomal protein L33	MRPL33	N98607	-4.0*
6h	32907_at	5-hydroxytryptamine (serotonin) receptor 6	HTR6	L41147	-2.5*
48h	40702_at	interferon, gamma	IFNG	X13274	113.4
	1021_at	interferon, gamma	IFNG	J00219	35.0
	1611_s_at	interferon, gamma	IFNG	J00219	28.1
	33093_at	interleukin 18 receptor accessory protein	IL18RAP	AF077346	12.0
	37137_at	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	GZMB	M17016	11.4
	32370_at	cytotoxic T-lymphocyte-associated serine esterase 1	CTLA1	M57888	5.6
	35338_at	paired basic amino acid cleaving enzyme (furin, membrane associated receptor protein)	PACE	X17094	3.9
	38326_at	putative lymphocyte G0/G1 switch gene	G0S2	M69199	3.4
	38278_at	modulator recognition factor I	MRF-1	M62324	3.4
	33305_at	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1	SERPINB1	M93056	3.2
	1004_at	Burkitt lymphoma receptor 1, GTP-binding protein	BLR1	X68149	2.6
	36776_at	lymphocyte-activation gene 3	LAG3	X51985	2.6
	40635_at	flotillin 1	FLOT1	AF089750	2.5
	1534_at	interleukin 12 receptor, beta 2	IL12RB2	U64198	2.3
	33513_at	signaling lymphocytic activation molecule	SLAM	U33017	2.2
	34654_at	myotubularin related protein 1	MTMR1	AJ224979	2.1
	38620_at	golgi SNAP receptor complex member 2	GOSR2	AA905543	-2.3*
	37623_at	nuclear receptor subfamily 4, group A, member 2	NR4A2	X75918	-2.5
	34866_at			AF055029	-2.8
	31856_at	glycoprotein A repetitions predominant	GARP	Z24680	-2.8
	39582_at			AL050166	-2.9*
	38575_at		MALT1	AF070528	-3.0*
	31496_g_at	small inducible cytokine subfamily C, member 2	SCYC2	D63789	-3.1
	33541_s_at	leukocyte-associated Ig-like receptor 2	LAIR2	AA133246	-3.4
	31540_at	Tumor necrosis factor receptor superfamily, member 9	TNFRSF9	U03397	-3.5

Table 1 (continued)

Target genes of IL-4 in human CD4+ T cells (Th2-induced vs activated)

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
2h	38549_at	vipirin	**cig5**	AF026941	**13.5**
	35659_at	interleukin 10 receptor, alpha	**IL10RA**	U00672	**6.5**
	974_at	mitogen-activated protein kinase kinase kinase 14	**MAP3K14**	Y10256	**5.1**
	34757_at	ADP-ribosyltransferase (NAD+; poly(ADP-ribose) polymerase)-like 2	**ADPRTL2**	AA595596	**4.6***
	1062_g_at	interleukin 10 receptor, alpha	**IL10RA**	U00672	**4.1**
	37544_at	nuclear factor, interleukin 3 regulated	**NFIL3**	X64318	**4.0**
	41193_at	dual specificity phosphatase 6	**DUSP6**	AB013382	**4.0**
	39593_at	Homo sapiens, Similar to fibrinogen-like 2, clone MGC:22391 IMAGE:4616866, mRNA, complete cds	.	AI432401	**3.9**
	587_at	endothelial differentiation, sphingolipid G-protein-coupled receptor, 1	**EDG1**	M31210	**3.4**
	38149_at	KIAA0053 gene product	**KIAA0053**	D29642	**3.2**
	1816_at	RAS p21 protein activator 2	**RASA2**	D78156	**3.2**
	41592_at	JAK binding protein	**SSI-1**	AB000734	**3.2**
	35712_at	leucine-rich repeat protein, neuronal 3	**LRRN3**	AC004142	**3.0**
	41384_at	receptor-interacting serine-threonine kinase 2	**RIPK2**	AF117829	**2.9**
	33047_at	ESTs, Weakly similar to B34087 hypothetical protein [H.sapiens]	.	AI971169	**2.9**
	33809_at	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1	**GNAI1**	AL049933	**2.8**
	38051_at	mal, T-cell differentiation protein	**MAL**	X76220	**2.7**
	828_at	prostaglandin E receptor 2 (subtype EP2), 53kD	**PTGER2**	U19487	**2.7**
	40456_at	up-regulated by BCG-CWS	**LOC64116**	AL049963	**2.7**
	1061_at	interleukin 10 receptor, alpha	**IL10RA**	U00672	**2.6**
	33291_at	RAS guanyl releasing protein 1 (calcium and DAG-regulated)	**RASGRP1**	AF081195	**2.5**
	36736_f_at	phosphoserine phosphatase	**PSPH**	Y10275	**2.5***
	37889_at	CD47 antigen (Rh-related antigen, integrin-associated signal transducer)	**CD47**	AA535376	**2.4***
	33260_at	son of sevenless homolog 1 (Drosophila)	**SOS1**	L13857	**2.3**
	35320_at	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2	**SLC11A2**	AB004857	**2.3**
	36899_at	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)	**SATB1**	M97287	**2.3**
	725_l_at	Chorionic Somatomammotropin Hormone Cs-5	.	HG1751-HT1768	**2.3***
	40511_at	GATA binding protein 3	**GATA3**	X58072	**2.3**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	34822_at	tumor protein p53 binding protein, 2	**TP53BP2**	U58334	**2.1**
	41145_at	KIAA0914 gene product	**KIAA0914**	AB020721	**2.1***
	41504_s_at	v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)	**MAF**	AF055376	**2.1**
	1860_at	tumor protein p53 binding protein, 2	**TP53BP2**	U58334	**2.0**
	649_s_at	chemokine (C-X-C motif), receptor 4 (fusin)	**CXCR4**	L06797	**2.0**
	39827_at	HIF-1 responsive RTP801	**RTP801**	AA522530	**-2.1**
	41475_at	ninjurin 1	**NINJ1**	U91512	**-2.3**
	33997_at	Homo sapiens mRNA; cDNA DKFZp586B1722 (from clone DKFZp586B1722)	**.**	AL049449	**-2.8***
	37043_at	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	**ID3**	AL021154	**-2.8**
6h	1062_g_at	interleukin 10 receptor, alpha	**IL10RA**	U00672	**12.6**
	41193_at	dual specificity phosphatase 6	**DUSP6**	AB013382	**8.6**
	38549_at	vipirin	**cig5**	AF026941	**7.7**
	41592_at	JAK binding protein	**SSI-1**	AB000734	**7.5**
	34348_at	serine protease inhibitor, Kunitz type, 2	**SPINT2**	U78095	**6.7**
	40511_at	GATA binding protein 3	**GATA3**	X58072	**4.9**
	1061_at	interleukin 10 receptor, alpha	**IL10RA**	U00672	**4.6**
	39330_s_at	actinin, alpha 1	**ACTN1**	M95178	**3.9**
	37121_at	natural killer cell group 7 sequence	**NKG7**	S69115	**3.6***
	34173_s_at	contactin 5	**CNTN5**	AB013802	**3.4***
	37544_at	nuclear factor, interleukin 3 regulated	**NFIL3**	X64318	**3.4**
	34319_at	S100 calcium binding protein P	**S100P**	AA131149	**3.1**
	37038_at	ATP-binding cassette, sub-family D (ALD), member 3	**ABCD3**	X83467	**3.0**
	931_at	Epstein-Barr virus induced gene 2 (lymphocyte-specific G protein-coupled receptor)	**EBI2**	L08177	**2.9**
	40839_at	ubiquitin-like 3	**UBL3**	AL080177	**2.7**
	33352_at	H2B histone family, member Q	**H2BFQ**	X57985	**2.6***
	37403_at	annexin A1	**ANXA1**	X05908	**2.6**
	39239_at	CD8 antigen, beta polypeptide 1 (p37)	**CD8B1**	X13444	**2.5**
	1067_at	fms-related tyrosine kinase 3 ligand	**FLT3LG**	U03858	**2.5***
	41577_at	protein phosphatase 1, regulatory (inhibitor) subunit 16B	**PPP1R16B**	AB020630	**2.5**
	1545_g_at	zyxin	**ZYX**	S77812	**2.4***
	31496_g_at	small inducible cytokine subfamily C, member 1 (lymphotactin)	**SCYC1**	D63789	**2.4**
	649_s_at	chemokine (C-X-C motif), receptor 4 (fusin)	**CXCR4**	L06797	**2.3**
	38051_at	mal, T-cell differentiation protein	**MAL**	X76220	**2.2**
	333_s_at	Single-Stranded Dna-Binding Protein Mssp-1	**.**	HG2639-HT2735	**2.1**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	37603_at	Interleukin 1 receptor antagonist	IL1RN	X52015	2.1
	34256_at	sialyltransferase 9 (CMP-NeuAc:lactosylceramide alpha-2,3-sialyltransferase; GM3 synthase)	SIAT9	AB018356	-2.1*
	34342_s_at	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	SPP1	AF052124	-2.1
	40385_at	small inducible cytokine subfamily A (Cys-Cys), member 20	SCYA20	U64197	-2.3
	41209_at	lipoprotein lipase	LPL	M15856	-2.3
	34375_at	small inducible cytokine A2 (monocyte chemotactic protein 1)	SCYA2	M28225	-2.5
	41744_at	optineurin	OPTN	AF070533	-2.5*
	32649_at	transcription factor 7 (T-cell specific, HMG-box)	TCF7	X59871	-2.6
	2092_s_at	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	SPP1	J04765	-2.8
	36239_at	POU domain, class 2, associating factor 1	POU2AF1	Z49194	-2.9
	875_g_at	small inducible cytokine A2 (monocyte chemotactic protein 1)	SCYA2	M26683	-3.1
	38578_at	Tumor necrosis factor receptor superfamily, member 7	TNFRSF7	M63928	-3.1
	37043_at	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	ID3	AL021154	-4.4
	33540_at	Homo sapiens mRNA; cDNA DKFZp564A023 (from clone DKFZp564A023)	.	AL049233	-4.6*
	39586_at	desmoglein 1	DSG1	AF097935	-4.6*
48h	37137_at	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	GZMB	M17016	10.8
	41504_s_at	v-maf musculoaponeurotic fibrosarcoma (avian) oncogene homolog	MAF	AF055376	8.9
	1892_s_at	neurotrophic tyrosine kinase, receptor, type 1	Trk	HG1437-HT1437	8.8
	41193_at	dual specificity phosphatase 6	DUSP6	AB013382	8.7
	34439_at	absent in melanoma 2	AIM2	AF024714	7.9
	1069_at	cyclooxygenase-2	Cox-2	U04636	6.6*
	41592_at	JAK binding protein	SSI-1	AB000734	6.0
	37038_at	peroxisomal membrane protein-1	ABCD3	X83467	5.9
	41505_r_at	v-maf musculoaponeurotic fibrosarcoma (avian) oncogene homolog	MAF	AF055376	5.9*
	35938_at	phospholipase A2, group IVA (cytosolic, calcium-dependent)	PLA2G4A	M72393	5.7
	37544_at	nuclear factor, interleukin 3 regulated	NFIL3	X64318	5.4
	40511_at	GATA-binding protein 3	GATA3	X58072	4.9
	36805_s_at	neurotrophic tyrosine kinase, receptor, type 1	NTRK1	X03541	4.7
	931_at	Epstein-Barr virus induced gene 2 (lymphocyte-specific G protein-coupled receptor)	EBI2	L08177	4.7
	41772_at	monoamine oxidase A	MAOA	M68840	4.1
	1368_at	interleukin 1 receptor, type I	IL1R1	M27492	3.9

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	34678_at	fer-1-like 3, myoferlin	**FER1L3**	AL096713	**3.7***
	37121_at	natural killer cell group 7 sequence	**NKG7**	S69115	**3.5**
	36899_at	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)	**SATB1**	M97287	**3.4**
	39942_at	basic leucine zipper transcription factor, ATF-like	**B-ATF**	AF016898	**3.2**
	38336_at	KIAA1013 protein	**KIAA1013**	AB023230	**3.1**
	41215_s_at	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	**ID2**	D13891	**2.7**
	1062_g_at	interleukin 10 receptor, alpha	**IL10RA**	U00672	**2.7**
	33305_at	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1	**SERPINB1**	M93056	**2.6**
	40848_g_at	KIAA0750 gene product	**KIAA0750**	AB018293	**2.5**
	37524_at	serine/threonine kinase 17b (apoptosis-inducing)	**STK17B**	AB011421	**2.4**
	34517_at	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	**HMGCS1**	X66435	**2.4**
	34348_at	serine protease inhibitor, Kunitz type, 2	**SPINT2**	U78095	**2.3**
	40448_at	zinc finger protein 36, C3H type, homolog (mouse)	**ZFP36**	M92843	**2.2**
	37732_at		**RYBP**	AL049940	**2.2**
	37616_at	AU RNA-binding protein/enoyl-Coenzyme A hydratase	**AUH**	X79888	**2.2**
	2002_s_at	BCL2-related protein A1	**BCL2A1**	U27467	**2.0**
	37842_at	I-mfa domain-containing protein	**.**	AF054589	**-2.0**
	37536_at	CD83 antigen (activated B lymphocytes, immunoglobulin superfamily)	**CD83**	Z11697	**-2.2**
	35735_at	guanylate binding protein 1, interferon-inducible, 67kD	**GBP1**	M55542	**-2.4**
	32859_at	signal transducer and activator of transcription 1, 91kD	**STAT1**	M97935	**-2.4**
	33705_at	phosphodiesterase 4B, cAMP-specific (dunce (Drosophila)-homolog phosphodiesterase E4)	**PDE4B**	L20971	**-2.6***
	33339_g_at	signal transducer and activator of transcription 1	**ISGF3**	M97936	**-2.9**
	38575_at		**MALT1**	AF070528	**-3.0***
	33338_at	signal transducer and activator of transcription 1	**ISGF3**	M97936	**-3.2**
	37623_at	nuclear receptor subfamily 4, group A, member 2	**NR4A2**	X75918	**-3.3**
	40729_s_at	lymphotoxin beta (TNF superfamily, member 3)	**LTB**	Y14768	**-3.4**
	37014_at	myxovirus (influenza) resistance 1, homolog of murine (interferon-inducible protein p78)	**MX1**	M33882	**-3.5**
	38326_at	putative lymphocyte G0/G1 switch gene	**G0S2**	M69199	**-3.8**
	547_s_at	nuclear receptor subfamily 4, group A, member 2	**NR4A2**	S77154	**-4.6**

TABLE 1 (continued)

Differences between Th1-induced vs Th2-induced human CD4+ T cells

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
2h	37043_at	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	**ID3**	AL021154	**2.5**
	37909_at	laminin, alpha 3 (nicein (150kD), kalinin (165kD), BM600 (150kD), epilegrin)	**LAMA3**	L34155	**2.4**
	36160_s_at	protein tyrosine phosphatase, receptor type, N polypeptide 2	**PTPRN2**	U81561	**2.3***
	39827_at	HIF-1 responsive RTP801	**RTP801**	AA522530	**2.1**
	38578_at	tumor necrosis factor receptor superfamily, member 7	**TNFRSF7**	M63928	**2.1**
	41475_at	ninjurin 1	**NINJ1**	U91512	**2.0**
	1860_at	tumor protein p53 binding protein, 2	**TP53BP2**	U58334	**-2.0**
	40511_at	GATA binding protein 3	**GATA3**	X58072	**-2.1**
	35320_at	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2	**SLC11A2**	AB004857	**-2.1**
	37524_at	serine/threonine kinase 17b (apoptosis-inducing)	**STK17B**	AB011421	**-2.2**
	32919_at	Homo sapiens, clone IMAGE:3625286, mRNA, partial cds	**.**	AC004010	**-2.3***
	1061_at	interleukin 10 receptor, alpha	**IL10RA**	U00672	**-2.3**
	39549_at	hypothetical protein FLJ23138	**FLJ23138**	AI743090	**-2.3***
	33352_at	H2B histone family, member Q	**H2BFQ**	X57985	**-2.4**
	36899_at	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)	**SATB1**	M97287	**-2.5**
	33260_at	son of sevenless homolog 1 (Drosophila)	**SOS1**	L13857	**-2.5**
	758_at	prostaglandin I2 (prostacyclin) receptor (IP)	**PTGIR**	D38128	**-2.5***
	1816_at	RAS p21 protein activator 2	**RASA2**	D78156	**-2.5**
	34398_at	heat shock 105kD	**HSP105B**	D86956	**-2.6***
	38051_at	mal, T-cell differentiation protein	**MAL**	X76220	**-2.6**
	33291_at	RAS guanyl releasing protein 1 (calcium and DAG-regulated)	**RASGRP1**	AF081195	**-2.6**
	34348_at	serine protease inhibitor, Kunitz type, 2	**SPINT2**	U78095	**-2.6**
	35712_at	leucine-rich repeat protein, neuronal 3	**LRRN3**	AC004142	**-2.6**
	40456_at	up-regulated by BCG-CWS	**LOC64116**	AL049963	**-2.8**
	41592_at	JAK binding protein	**SSI-1**	AB000734	**-2.9**
	38149_at	KIAA0053 gene product	**KIAA0053**	D29642	**-2.9**
	33809_at	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1	**GNAI1**	AL049933	**-2.9**
	36435_at	protein phosphatase 1A (formerly 2C), magnesium-dependent, alpha isoform	**PPM1A**	AF070670	**-3.0**
	32058_at	HNK-1 sulfotransferase	**HNK-1ST**	AF070594	**-3.0**
	36148_at	amyloid beta (A4) precursor-like protein 1	**APLP1**	U48437	**-3.5***

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	41193_at	dual specificity phosphatase 6	**DUSP6**	AB013382	**-3.7**
	41384_at	receptor-interacting serine-threonine kinase 2	**RIPK2**	AF117829	**-3.9**
	33047_at	ESTs, Weakly similar to B34087 hypothetical protein [H.sapiens]	**.**	AI971169	**-4.0**
	1062_g_at	interleukin 10 receptor, alpha	**IL10RA**	U00672	**-4.0**
	587_at	endothelial differentiation, sphingolipid G-protein-coupled receptor, 1	**EDG1**	M31210	**-4.1**
	32148_at	FERM, RhoGEF (ARHGEF) and pleckstrin domain protein 1 (chondrocyte-derived)	**FARP1**	AI701049	**-4.3***
	37544_at	nuclear factor, interleukin 3 regulated	**NFIL3**	X64318	**-5.1**
	974_at	mitogen-activated protein kinase kinase kinase 14	**MAP3K14**	Y10256	**-5.3**
	35659_at	interleukin 10 receptor, alpha	**IL10RA**	U00672	**-5.7**
	39593_at	Homo sapiens, Similar to fibrinogen-like 2, clone MGC:22391 IMAGE:4616866, mRNA, complete cds	**.**	AI432401	**-9.2**
	38549_at	vipirin	**cig5**	AF026941	**-35.5**
6h	40085_s_at	transcription factor CP2	**TFCP2**	U03495	**9.2***
	40757_at	granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)	**GZMA**	M18737	**3.6**
	32041_r_at	CDC5 cell division cycle 5-like (S. pombe)	**CDC5L**	AB007892	**3.5***
	36705_at	protein kinase, AMP-activated, beta 2 non-catalytic subunit	**PRKAB2**	AJ224538	**3.1***
	32649_at	transcription factor 7 (T-cell specific, HMG-box)	**TCF7**	X59871	**2.9**
	40091_at	B-cell CLL/lymphoma 6 (zinc finger protein 51)	**BCL6**	U00115	**2.8**
	875_g_at	small inducible cytokine A2 (monocyte chemotactic protein 1)	**SCYA2**	M26683	**2.8**
	36239_at	POU domain, class 2, associating factor 1	**POU2AF1**	Z49194	**2.7**
	38578_at	tumor necrosis factor receptor superfamily, member 7	**TNFRSF7**	M63928	**2.7**
	34252_at	hypothetical protein FLJ10342	**FLJ10342**	W28545	**2.6***
	35735_at	guanylate binding protein 1, interferon-inducible, 67kD	**GBP1**	M55542	**2.4**
	39389_at	CD9 antigen (p24)	**CD9**	M38690	**2.4**
	40702_at	interferon, gamma	**IFNG**	X13274	**2.3**
	1369_s_at	interleukin 8	**IL8**	M28130	**2.2**
	37485_at	fatty-acid-Coenzyme A ligase, very long-chain 1	**FACVL1**	D88308	**2.2**
	40698_at	C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 2 (activation-induced)	**CLECSF2**	X96719	**2.2**
	1954_at	kinase insert domain receptor (a type III receptor tyrosine kinase)	**KDR**	AF035121	**2.0***
	34256_at	sialyltransferase 9 (CMP-NeuAc:lactosylceramide alpha-2,3-sialyltransferase; GM3 synthase)	**SIAT9**	AB018356	**2.0***
	37025_at	LPS-induced TNF-alpha factor	**PIG7**	AL120815	**2.0**
	35693_at	hippocalcin-like 1	**HPCAL1**	AF070616	**-2.0**
	38051_at	mal, T-cell differentiation protein	**MAL**	X76220	**-2.1**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	558_at	keratin 1 (epidermolytic hyperkeratosis)	**KRT1**	M98776	**-2.1***
	34319_at	S100 calcium binding protein P	**S100P**	AA131149	**-2.2**
	37403_at	annexin A1	**ANXA1**	X05908	**-2.3**
	31496_g_at	small inducible cytokine subfamily C, member 1 (lymphotactin)	**SCYC1**	D63789	**-2.4**
	37038_at	ATP-binding cassette, sub-family D (ALD), member 3	**ABCD3**	X83467	**-2.4**
	40839_at	ubiquitin-like 3	**UBL3**	AL080177	**-2.4**
	35794_at	KIAA0942 protein	**KIAA0942**	AB023159	**-2.5***
	39330_s_at	actinin, alpha 1	**ACTN1**	M95178	**-2.5**
	974_at	mitogen-activated protein kinase kinase kinase 14	**MAP3K14**	Y10256	**-2.6**
	37544_at	nuclear factor, interleukin 3 regulated	**NFIL3**	X64318	**-2.8**
	37398_at	platelet/endothelial cell adhesion molecule (CD31 antigen)	**PECAM1**	AA100961	**-2.9***
	931_at	Epstein-Barr virus induced gene 2 (lymphocyte-specific G protein-coupled receptor)	**EBI2**	L08177	**-2.9**
	1061_at	interleukin 10 receptor, alpha	**IL10RA**	U00672	**-3.1**
	37121_at	natural killer cell group 7 sequence	**NKG7**	S69115	**-3.5**
	39239_at	CD8 antigen, beta polypeptide 1 (p37)	**CD8B1**	X13444	**-3.5**
	35422_at	microtubule-associated protein 2	**MAP2**	U01828	**-3.6***
	40511_at	GATA binding protein 3	**GATA3**	X58072	**-4.0**
	34990_at	SET binding protein 1	**SETBP1**	AB022660	**-4.4***
	41592_at	JAK binding protein	**SSI-1**	AB000734	**-5.3**
	34348_at	serine protease inhibitor, Kunitz type, 2	**SPINT2**	U78095	**-5.9**
	38549_at	vipirin	**cig5**	AF026941	**-6.1**
	1062_g_at	interleukin 10 receptor, alpha	**IL10RA**	U00672	**-6.3**
	41193_at	dual specificity phosphatase 6	**DUSP6**	AB013382	**-6.7**
48h	40702_at	interferon, gamma	**IFNG**	X13274	**115.4**
	1021_at	interferon, gamma	**IFNG**	J00219	**48.8**
	33093_at	interleukin 18 receptor accessory protein	**IL18RAP**	AF077346	**18.4**
	1611_s_at	interferon, gamma	**IFNG**	J00219	**18.2**
	38326_at	putative lymphocyte G0/G1 switch gene	**G0S2**	M69199	**10.6**
	1538_s_at	interleukin 2	**IL2**	X00695	**7.3**
	31792_at	annexin A3	**ANXA3**	M20560	**6.0**
	39264_at	2'-5'-oligoadenylate synthetase 2 (69-71 kD)	**OAS2**	M87284	**4.0***
	34021_at	interleukin 2	**IL2**	S82692	**3.5**
	32370_at	cytotoxic T-lymphocyte-associated serine esterase 1	**CTLA1**	M57888	**3.2**
	31961_r_at		**.**	AF070579	**2.8***
	38463_s_at	adenosine monophosphate deaminase (isoform E)	**AMPD3**	U29926	**2.5***

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	34607_at	inducible T-cell co-stimulator	**ICOS**	AB023135	**2.3**
	40396_at	purinergic receptor P2X, ligand-gated ion channel, 5	**P2RX5**	U49395	**2.2***
	1004_at	Burkitt lymphoma receptor 1, GTP-binding protein	**BLR1**	X68149	**2.1***
	441_s_at	Leukemia inhibitory factor (cholinergic differentiation factor)	**LIF**	X13967	**2.0***
	34678_at	fer-1-like 3, myoferlin	**FER1L3**	AL096713	**-2.1***
	35712_at	leucine-rich repeat protein, neuronal 3	**LRRN3**	AC004142	**-2.3**
	34091_s_at	vimentin	**VIM**	Z19554	**-2.3**
	40456_at		**.**	AL049963	**-2.3**
	33541_s_at	leukocyte-associated Ig-like receptor 2	**LAIR2**	AA133246	**-2.4**
	1061_at	interleukin 10 receptor, alpha	**IL10RA**	U00672	**-2.4**
	36899_at	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)	**SATB1**	M97287	**-2.5**
	36926_at	mitogen-activated protein kinase 6	**MAPK6**	X80692	**-2.6***
	649_s_at	chemokine (C-X-C motif), receptor 4 (fusin)	**CXCR4**	L06797	**-2.7**
	38685_at	hypothetical protein MGC14797	**MGC14797**	AL035306	**-2.8***
	34348_at	serine protease inhibitor, Kunitz type, 2	**SPINT2**	U78095	**-3.0**
	40049_at	death-associated protein kinase 1	**DAPK1**	X76104	**-3.0***
	37544_at	nuclear factor, interleukin 3 regulated	**NFIL3**	X64318	**-3.1**
	39331_at	tubulin, beta polypeptide	**TUBB**	X79535	**-3.2***
	31856_at	glycoprotein A repetitions predominant	**GARP**	Z24680	**-3.6**
	931_at	Epstein-Barr virus induced gene 2 (lymphocyte-specific G protein-coupled receptor)	**EBI2**	L08177	**-3.8**
	41504_s_at	v-maf musculoaponeurotic fibrosarcoma (avian) oncogene homolog	**MAF**	AF055376	**-4.0**
	41193_at	dual specificity phosphatase 6	**DUSP6**	AB013382	**-4.1**
	494_at	interleukin 13	**IL-13**	U31120	**-4.3***
	1069_at		**Cox-2**	U04636	**-4.8***
	33809_at	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1	**GNAI1**	AL049933	**-4.9**
	1062_g_at	interleukin 10 receptor, alpha	**IL10RA**	U00672	**-5.2**
	37038_at	peroxisomal membrane protein-1	**ABCD3**	X83467	**-5.7**
	41592_at	JAK binding protein	**SSI-1**	AB000734	**-7.2**
	36805_s_at	neurotrophic tyrosine kinase, receptor, type 1	**NTRK1**	X03541	**-8.2**
	40511_at	GATA-binding protein 3	**GATA3**	X58072	**-8.8**
	35938_at	phospholipase A2, group IVA (cytosolic, calcium-dependent)	**PLA2G4A**	M72393	**-10.5***
	41772_at	monoamine oxidase A	**MAOA**	M68840	**-11.1**
	1892_s_at	neurotrophic tyrosine kinase, receptor, type 1	**Trk**	HG1437-HT1437	**-12.2**

TABLE 1 (continued)

Target genes of TGFb in human CD4+ T cells cultured in Th1 conditions (Th1-induced +TGFb vs Th1-induced))

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
2h	41049_at	insulin receptor substrate 1	IRS1	S62539	3.7*
	34414_at	KIAA0368 protein	KIAA0368	AB002366	2.9*
	37909_at	laminin, alpha 3 (nicein (150Kd), kalinin (165Kd), BM600 (150Kd), epilegrin)	LAMA3	L34155	2.2
	39822_s_at	growth arrest and DNA-damage-inducible, beta	GADD45B	AF078077	2.0
	32870_g_at	MRE11 meiotic recombination 11 homolog A (S. cerevisiae)	MRE11A	AF073362	-2.4*
	32639_at	nucleoporin-like protein 1	NLP_1	U97198	-2.5*
	33042_r_at	Homo sapiens clone 24442 Mrna sequence	.	AF055018	-3.1*
	38645_at	DKFZP434A043 protein	DKFZP434A043	AL096748	-3.4*
6h	41779_at	regulator of G-protein ignaling 16	RGS16	U70426	7.2
	40923_at	Homo sapiens Cdna: FLJ21449 fis, clone COL04483, highly similar to AF010235 Homo sapiens Mrna from chromosome 5q31-33 region	.	AA290994	5.3*
	38325_at	multiple inositol polyphosphate histidine phosphatase, 1	MINPP1	AL050356	4.9
	37391_at	cathepsin L	CTSL	X12451	3.9
	35338_at	paired basic amino acid cleaving enzyme (furin, membrane associated receptor protein)	PACE	X17094	3.1
	32583_at	v-jun sarcoma virus 17 oncogene homolog (avian)	JUN	J04111	3.0
	37669_s_at	ATPase, Na+/K+ transporting, beta 1 polypeptide	ATP1B1	U16799	3.0
	1388_g_at	vitamin D (1,25- dihydroxyvitamin D3) receptor	VDR	J03258	2.9
	2031_s_at	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	CDKN1A	U03106	2.5
	40385_at	small inducible cytokine subfamily A (Cys-Cys), member 20	SCYA20	U64197	2.5
	1830_s_at	transforming growth factor, beta 1 (Camurati-Engelmann disease)	TGFB1	M38449	2.5
	38077_at	collagen, type VI, alpha 3	COL6A3	X52022	2.5
	41438_at	oxysterol binding protein-like 8	OSBPL8	AL049923	2.4
	32607_at	Brain abundant, membrane attached signal protein 1	BASP1	AF039656	2.3
	33812_at	a disintegrin and metalloproteinase domain 19 (meltrin beta)	ADAM19	AL049415	2.3
	39071_at	integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)	ITGAV	M14648	2.2
	39827_at	HIF-1 responsive RTP801	RTP801	AA522530	2.1
	37043_at	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	ID3	AL021154	2.1
	1857_at	MAD, mothers against decapentaplegic homolog 7 (Drosophila)	MADH7	AF010193	2.0*
	37279_at	GTP binding protein overexpressed in skeletal muscle	GEM	U10550	2.0

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	33575_at	adhesion glycoprotein	**DNAM-1**	U56102	**-2.1**
	2017_s_at	cyclin D1 (PRAD1: parathyroid adenomatosis 1)	**CCND1**	M64349	**-2.2**
	40310_at	toll-like receptor 2	**TLR2**	AF051152	**-2.7***
	33715_r_at	general transcription factor IIH, polypeptide 2 (44Kd subunit)	**GTF2H2**	U80017	**-2.7***
	31324_at			U82303	**-2.8***
	35249_at	cyclin E2	**CCNE2**	AF091433	**-3.9***
48h	1405_i_at	small inducible cytokine A5 (RANTES)	**SCYA5**	M21121	**11.8**
	1403_s_at	small inducible cytokine A5 (RANTES)	**SCYA5**	M21121	**7.6**
	40385_at	small inducible cytokine subfamily A (Cys-Cys), member 20	**SCYA20**	U64197	**4.9**
	34091_s_at	vimentin	**VIM**	Z19554	**4.5**
	38391_at	capping protein (actin filament), gelsolin-like	**CAPG**	M94345	**4.3**
	41209_at	lipoprotein lipase	**LPL**	M15856	**4.1**
	41472_at	phorbolin-like protein MDS019	**MDS019**	AL078641	**3.9**
	37669_s_at	ATPase, Na+/K+ transporting, beta 1 polypeptide	**ATP1B1**	U16799	**3.9**
	1788_s_at	dual specificity phosphatase 4	**DUSP4**	U48807	**3.8**
	34947_at	phorbolin-like protein MDS019	**MDS019**	AA442560	**3.3**
	32607_at	Brain abundant, membrane attached signal protein 1	**BASP1**	AF039656	**3.1**
	31540_at	Tumor necrosis factor receptor superfamily, member 9	**TNFRSF9**	U03397	**3.0**
	33232_at	cysteine-rich protein 1 (intestinal)	**CRIP1**	AI017574	**2.7**
	40662_g_at	nuclear receptor subfamily 4, group A, member 3	**NR4A3**	D78579	**2.5***
	2069_s_at	catenin (cadherin-associated protein), alpha 1 (102Kd)	**CTNNA1**	L23805	**2.5**
	41779_at	regulator of G-protein ignaling 16	**RGS16**	U70426	**2.5**
	769_s_at	annexin A2	**ANXA2**	D00017	**2.4**
	1358_s_at	interferon, alpha-inducible protein	**G1P3**	U22970	**2.2***
	33412_at	lectin, galactoside-binding, soluble, 1 (galectin 1)	**LGALS1**	AI535946	**2.2**
	31444_s_at	Annexin A2 pseudogene 3	**ANXA2P3**	M62895	**2.1**
	40365_at	guanine nucleotide binding protein (G protein), alpha 15 (Gq class)	**GNA15**	M63904	**-2.1**
	36545_s_at	KIAA0542 gene product	**KIAA0542**	AB011114	**-2.2**
	37137_at	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	**GZMB**	M17016	**-2.6**
	37544_at	nuclear factor, interleukin 3 regulated	**NFIL3**	X64318	**-3.3**
	33285_i_at	Hypothetical protein FLJ21168	**FLJ21168**	W26762	**-3.4***
	36899_at	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)	**SATB1**	M97287	**-3.5**
	38944_at	MAD (mothers against decapentaplegic, Drosophila) homolog 3	**MADH3**	U68019	**-7.2**

TABLE 1 (continued)

Target genes of TGFb in human CD4+ T cells cultured in Th2 conditions (Th2-induced +TGFb vs Th2-induced))

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
2h	33435_r_at	BET1 homolog (S. cerevisiae)	**BET1**	AI525962	**6.3***
	32709_at	potassium voltage-gated channel, shaker-related subfamily, beta member 1	**KCNAB1**	L39833	**4.0***
	40095_at	carbonic anhydrase II	**CA2**	J03037	**2.3**
	2031_s_at	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	**CDKN1A**	U03106	**2.0**
	32770_at	SEC24 related gene family, member D (S. cerevisiae)	**SEC24D**	AB018298	**-3.6***
6h	39260_at	solute carrier family 16 (monocarboxylic acid transporters), member 4	**SLC16A4**	U59185	**8.6***
	32583_at	v-jun sarcoma virus 17 oncogene homolog (avian)	**JUN**	J04111	**8.3**
	1388_g_at	vitamin D (1,25- dihydroxyvitamin D3) receptor	**VDR**	J03258	**4.4**
	41779_at	regulator of G-protein signalling 16	**RGS16**	U70426	**4.3**
	40647_at	Kell blood group precursor (McLeod phenotype)	**XK**	Z32684	**3.2***
	37391_at	cathepsin L	**CTSL**	X12451	**3.1**
	555_at	RAB40B, member RAS oncogene family	**RAB40B**	U05227	**3.1***
	40385_at	small inducible cytokine subfamily A (Cys-Cys), member 20	**SCYA20**	U64197	**3.0**
	33186_i_at	ESTs	**.**	AL046961	**2.9***
	38077_at	collagen, type VI, alpha 3	**COL6A3**	X52022	**2.5**
	41788_i_at	KIAA0669 gene product	**KIAA0669**	AB014569	**2.5**
	312_s_at		**.**	HG3075-HT3236	**2.5**
	35338_at	paired basic amino acid cleaving enzyme (furin, membrane associated receptor protein)	**PACE**	X17094	**2.4**
	36089_at	Sac domain-containing inositol phosphatase 2	**SAC2**	AB023183	**2.3**
	34800_at	ortholog of mouse integral membrane glycoprotein LIG-1	**LIG1**	AL039458	**2.2**
	33893_r_at	KIAA0470 gene product	**KIAA0470**	AB007939	**2.1**
	40790_at	basic helix-loop-helix domain containing, class B, 2	**BHLHB2**	AB004066	**2.1**
	37279_at	GTP binding protein overexpressed in skeletal muscle	**GEM**	U10550	**2.1**
	40399_r_at	mesenchyme homeo box 2 (growth arrest-specific homeo box)	**MEOX2**	AI743406	**-2.3***
	528_at	heat shock 27kD protein 3	**HSPB3**	U15590	**-3.9***
	41291_at	GDP dissociation inhibitor 2	**GDI2**	AC004528	**-8.0**
48h	37669_s_at	ATPase, Na+/K+ transporting, beta 1 polypeptide	**ATP1B1**	U16799	**11.5**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	1183_at	small inducible cytokine subfamily A (Cys-Cys), member 17	**SCYA17**	D43767	**10.1**
	1405_i_at	small inducible cytokine A5 (RANTES)	**SCYA5**	M21121	**8.5**
	977_s_at	cadherin 1, type 1, E-cadherin	**ECAD3**	Z35402	**8.1**
	35367_at	lectin, galactoside-binding, soluble, 3 (galectin 3)	**LGALS3**	AB006780	**5.9**
	1403_s_at	small inducible cytokine A5 (RANTES)	**SCYA5**	M21121	**5.4**
	32607_at	brain abundant, membrane attached signal protein 1	**BASP1**	AF039656	**4.1**
	34037_at	interleukin 9	**IL9**	M30134	**3.9**
	35824_at	zinc finger protein 238	**ZNF238**	AJ223321	**3.7***
	36575_at	regulator of G-protein signalling 1	**RGS1**	S59049	**3.7**
	36117_at	PTK2 protein tyrosine kinase 2	**PTK2**	L13616	**3.5**
	34739_at	hypothetical protein FLJ20275	**FLJ20275**	W26023	**3.4***
	37603_at	interleukin 1 receptor antagonist	**IL1RN**	X52015	**3.2**
	39248_at	aquaporin 3	**AQP3**	N74607	**3.1**
	34217_at	Kruppel-like factor 7 (ubiquitous)	**KLF7**	AB015132	**2.5**
	37377_i_at	lamin A/C	**LMNA**	M13452	**2.4**
	40818_at	H-2K binding factor-2	**LOC51580**	D14041	**2.4**
	37391_at	cathepsin L	**CTSL**	X12451	**2.3***
	32587_at	zinc finger protein 36, C3H type-like 2	**ZFP36L2**	U07802	**2.2***
	32778_at	inositol 1,4,5-triphosphate receptor, type 1	**ITPR1**	D26070	**-2.1***
	38006_at	CD48 antigen (B-cell membrane protein)	**CD48**	M37766	**-2.2**
	41215_s_at	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	**ID2**	D13891	**-2.2**
	33849_at	pre-B-cell colony-enhancing factor	**PBEF**	U02020	**-2.3**
	34801_at	KIAA0710 gene product	**KIAA0710**	AB014610	**-2.4***
	34098_f_at	integrin cytoplasmic domain-associated protein 1	**ICAP-1A**	AI799757	**-2.5**
	36377_at	interleukin 18 receptor 1	**IL18R1**	U43672	**-2.5**
	1069_at	cyclooxygenase-2	**Cox-2**	U04636	**-2.5***
	38122_at	solute carrier family 23 (nucleobase transporters), member 1	**SLC23A1**	D87075	**-2.6***
	35943_s_at	GA-binding protein transcription factor, beta subunit 1 (53kD)	**GABPB1**	D13317	**-2.8***
	2002_s_at	BCL2-related protein A1	**BCL2A1**	U27467	**-2.9**
	33809_at	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1	**GNAI1**	AL049933	**-2.9***
	34965_at	cystatin F (leukocystatin)	**CST7**	AF031824	**-3.0**
	37544_at	nuclear factor, interleukin 3 regulated	**NFIL3**	X64318	**-3.0**
	36160_s_at	protein tyrosine phosphatase, receptor type, N polypeptide 2	**PTPRN2**	U81561	**-3.1**
	35938_at	phospholipase A2, group IVA (cytosolic, calcium-dependent)	**PLA2G4A**	M72393	**-4.0**

*Gene was classified to be "not changed" by Affymetrix MAS5 software in either one or in both biological replicates.

TABLE 1 (continued)

Summary of data on target genes of IL-12 at 48 h in human CD4+ T cells (Th1-induced vs activated)

Probe_ID	RL3_Signal Log Ratio	RL4_Signal Log Ratio	Accession	Gene name	Gene symbol	Locus	Functional group
40702_at	8.43	5.22	X13274	interferon, gamma	IFNG	12q14	Cytokines, chemokines and other ligands
1021_at	5.26	5	J00219	interferon, gamma	IFNG	12q14	Cytokines, chemokines and other ligands
1611_s_at	6.07	3.55	J00219	interferon, gamma	IFNG	12q14	Cytokines, chemokines and other ligands
33093_at	2.95	4.22	AF077346	interleukin 18 receptor accessory protein	IL18RAP	2p24.3-p24.1	Cell surface molecule
37137_at	2.96	4.05	M17016	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	GZMB	14q11.2	Enzymes and other pathway molecules
32370_at	2.59	2.39	M57888	cytotoxic T-lymphocyte-associated serine esterase 1	CTLA1	14q11.2	Enzymes and other pathway molecules
35338_at	1.68	2.21	X17094	paired basic amino acid cleaving enzyme (furin, membrane associated receptor protein)	PACE	15q25-q26	Enzymes and other pathway molecules
38326_at	1.65	1.92	M69199	putative lymphocyte G0/G1 switch gene	G0S2	1	Enzymes and other pathway molecules
38278_at	1.47	2.09	M62324	modulator recognition factor I	MRF-1	2p11.1	Transcriptional regulator
33305_at	1.62	1.74	M93056	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1	SERPINB1	6p25	Enzymes and other pathway molecules
1004_at	1.75	1.05	X68149	Burkitt lymphoma receptor 1, GTP-binding protein	BLR1	11q23.3	Cell surface molecule
36776_at	1.33	1.41	X51985	lymphocyte-activation gene 3	LAG3	12p13.32	Cell surface molecule
40635_at	1.16	1.47	AF089750	flotillin 1	FLOT1	6p21.3	Unclassified
1534_at	1.05	1.38	U64198	interleukin 12 receptor, beta 2	IL12RB2	1p31.3-p31.2	Cell surface molecule
33513_at	1.13	1.08	U33017	signaling lymphocytic activation molecule	SLAM	1q22-q23	Cell surface molecule
34654_at	1.04	1.04	AJ224979	myotubularin related protein 1	MTMR1	Xq28	Enzymes and other pathway molecules
38620_at	-1.11	-1.33	AA905543	golgi SNAP receptor complex member 2	GOSR2	17q21	Structural and intracellular

Probe_ID	RL3_Signal Log Ratio	RL4_Signal Log Ratio	Accession	Gene name	Gene symbol	Locus	Functional group
							trafficing molecules
37623_at	-1.64	-1.05	X75918	nuclear receptor subfamily 4, group A, member 2	NR4A2	2q22-q23	Transcriptional regulator
34866_at	-1.37	-1.64	AF055029				Unclassified
31856_at	-1.92	-1.1	Z24680	glycoprotein A repetitions predominant	GARP	11q13.5-q14	Cell surface molecule
39582_at	-1.97	-1.12	AL050166				Unclassified
38575_at	-1.83	-1.37	AF070528				Unclassified
31496_g_at	-1.92	-1.38	D63789	small inducible cytokine subfamily C, member 2	SCYC2	1q23-q25	Cytokines, chemokines and other ligands
33541_s_at	-1.8	-1.71	AA133246	leukocyte-associated Ig-like receptor 2	LAIR2	19q13.4	Cell surface molecule
31540_at	-2.1	-1.51	U03397	tumor necrosis factor receptor superfamily, member 9	TNFRSF9	1p36	Cell surface molecule

TABLE 1 (continued)

Summary of data on target genes of IL-4 at 48 h in human CD4+ T cells (Th2-induced vs activated)

Probe_ID	RI3_Signal Log Ratio	RL4_Signal Log Ratio	Accession	Gene name	Gene symbol	Locus	Functional group
37137_at	2.73	4.14	M17016	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	GZMB	14q11.2	Enzymes and other pathway molecules
41504_s_at	2.82	3.49	AF055376	v-maf musculoaponeurotic fibrosarcoma (avian) oncogene homolog	MAF	16q22-q23	Transcriptional regulator
1892_s_at	2.90	3.36	HG1437-HT1437	neurotrophic tyrosine kinase, receptor, type 1	Trk	1q21-q22	Cell surface molecule
41193_at	2.52	3.71	AB013382	dual specificity phosphatase 6	DUSP6	12q22-q23	Enzymes and other pathway molecules
34439_at	2.58	3.39	AF024714	absent in melanoma 2	AIM2	1q22	Enzymes and other pathway molecules
1069_at	4.33	1.11	U04636	cyclooxygenase-2	Cox-2	1q25.2-q25.3	Enzymes and other pathway molecules
41592_at	2.70	2.45	AB000734	JAK binding protein	SSI-1	16p13.13	Enzymes and other pathway molecules
37038_at	2.63	2.48	X83467	peroxisomal membrane protein-1	PXMP1	1p22-p21	Structural and intracellular trafficing molecules
41505_r_at	2.54	2.56	AF055376	v-maf musculoaponeurotic fibrosarcoma (avian) oncogene homolog	MAF	16q22-q23	Transcriptional regulator
35938_at	3.70	1.30	M72393	phospholipase A2, group IVA (cytosolic, calcium-dependent)	PLA2G4A	1q25	Enzymes and other pathway molecules
37544_at	2.34	2.54	X64318	nuclear factor, interleukin 3 regulated	NFIL3	9q22	Transcriptional regulator
40511_at	2.53	2.04	X58072	GATA-binding protein 3	GATA3	10p15	Transcriptional regulator
36805_s_at	1.58	2.91	X03541	neurotrophic tyrosine kinase, receptor, type 1	NTRK1	1q21-q22	Cell surface molecule
931_at	2.63	1.85	L08177	Epstein-Barr virus induced gene 2 (lymphocyte-specific G protein-coupled receptor)	EBI2	13q32.3	Cell surface molecule
41772_at	2.16	1.93	M68840	monoamine oxidase A	MAOA	Xp11.4-p11.3	Enzymes and other pathway molecules
1368_at	1.92	1.99	M27492	interleukin 1 receptor, type I	IL1R1	2q12	Cell surface molecule
34678_at	1.81	1.98	AL096713	fer-1-like 3, myoferlin	FER1L3	10q24	Unclassified
37121_at	2.47	1.18	S69115	natural killer cell group 7 sequence	NKG7	19q13.41	Cell surface molecule

Probe_ID	RI3_Signal Log Ratio	RL4_Signal Log Ratio	Accession	Gene name	Gene symbol	Locus	Functional group
36899_at	1.49	2.02	M97287	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)	SATB1	3p23	Structural and intracellular trafficing molecules
39942_at	1.37	2.03	AF016898	basic leucine zipper transcription factor, ATF-like	B-ATF	14q24.3	Transcriptional regulator
38336_at	1.60	1.71	AB023230	KIAA1013 protein	KIAA1013	3	Unclassified
41215_s_at	1.56	1.34	D13891	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	ID2	2p25	Enzymes and other pathway molecules
1062_g_at	1.70	1.16	U00672	interleukin 10 receptor, alpha	IL10RA	11q23	Cell surface molecule
33305_at	1.20	1.58	M93056	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1	SERPINB1	6p25	Enzymes and other pathway molecules
40848_g_at	1.25	1.36	AB018293	KIAA0750 gene product	KIAA0750	11p15.3	Unclassified
37524_at	1.09	1.44	AB011421	serine/threonine kinase 17b (apoptosis-inducing)	STK17B	2q33.1	Enzymes and other pathway molecules
34517_at	1.16	1.36	X66435	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	HMGCS1	5p14-p13	Enzymes and other pathway molecules
34348_at	1.33	1.12	U78095	serine protease inhibitor, Kunitz type, 2	SPINT2	19q13.1	Enzymes and other pathway molecules
40448_at	1.02	1.26	M92843	zinc finger protein 36, C3H type, homolog (mouse)	ZFP36	19q13.1	Enzymes and other pathway molecules
37732_at	1.11	1.16	AL049940				Unclassified
37616_at	1.16	1.05	X79888	AU RNA-binding protein/enoyl-Coenzyme A hydratase	AUH	9q22.1	Enzymes and other pathway molecules
2002_s_at	1.01	1.03	U27467	BCL2-related protein A1	BCL2A1	15q24.3	Enzymes and other pathway molecules
37842_at	-1.02	-1.01	AF054589	I-mfa domain-containing protein	HIC	7q31.33	Unclassified
37536_at	-1.17	-1.10	Z11697	CD83 antigen (activated B lymphocytes, immunoglobulin superfamily)	CD83	6p23	Cell surface molecule
35735_at	-1.18	-1.32	M55542	guanylate binding protein 1, interferon-inducible, 67kD	GBP1	1p22.1	Unclassified
32859_at	-1.27	-1.29	M97935	signal transducer and activator of transcription 1, 91kD	STAT1	2q32.2	Transcriptional regulator
33705_at	-1.43	-1.37	L20971	phosphodiesterase 4B, cAMP-specific (dunce (Drosophila)-homolog phosphodiesterase E4)	PDE4B	1p31	Enzymes and other pathway molecules
33339_g_at	-1.04	-2.02	M97936	signal transducer and activator of transcription 1	ISGF3	2q32.2	Transcriptional regulator

Probe_ID	RI3_Signal Log Ratio	RL4_Signal Log Ratio	Accession	Gene name	Gene symbol	Locus	Functional group
38575_at	-1.16	-2.00	AF070528				Unclassified
33338_at	-1.28	-2.07	M97936	signal transducer and activator of transcription 1	ISGF3	2q32.2	Transcriptional regulator
37623_at	-1.74	-1.74	X75918	nuclear receptor subfamily 4, group A, member 2	NR4A2	2q22-q23	Transcriptional regulator
40729_s_at	-2.36	-1.13	Y14768	lymphotoxin beta (TNF superfamily, member 3)	LTB	6p21.3	Cytokines, chemokines and other ligands
37014_at	-1.02	-2.62	M33882	myxovirus (influenza) resistance 1, homolog of murine (interferon-inducible protein p78)	MX1	21q22.3	Enzymes and other pathway molecules
38326_at	-2.13	-1.74	M69199	putative lymphocyte G0/G1 switch gene	G0S2	1	Enzymes and other pathway molecules
547_s_at	-2.07	-2.34	S77154	nuclear receptor subfamily 4, group A, member 2	NR4A2	2q22-q23	Transcriptional regulator

TABLE 1 (continued)

Summary of data on genes differentially expressed at 48 h when gene expression of human CD4+ T cells induced to Th1 direction is compared to gene expression of human CD4+ T cells induced to Th2 direction.

Probe_ID	RL3_Signal Log Ratio	RI4_Signal Log Ratio	Accession	Gene name	Gene symbol	Locus	Functional group
40702_at	6.35	7.35	X13274	interferon, gamma	IFNG	12q14	Cytokines, chemokines and other ligands
33093_at	4.31	4.09	AF077346	interleukin 18 receptor accessory protein	IL18RAP	2p24.3-p24.1	Cell surface molecule
1021_at	6.16	5.06	J00219	interferon, gamma	IFNG	12q14	Cytokines, chemokines and other ligands
1611_s_at	5.03	3.34	J00219	interferon, gamma	IFNG	12q14	Cytokines, chemokines and other ligands
38326_at	3.50	3.31	M69199	putative lymphocyte G0/G1 switch gene	G0S2	1	Enzymes and other pathway molecules
31792_at	3.76	1.43	M20560	annexin A3	ANXA3	4q13-q22	Enzymes and other pathway molecules
39264_at	1.84	2.15	M87284	2'-5'-oligoadenylate synthetase 2 (69-71 kD)	OAS2	12q24.2	Enzymes and other pathway molecules
34021_at	1.43	2.21	S82692	interleukin 2	IL2	4q26-q27	Cytokines, chemokines and other ligands
32370_at	1.95	1.38	M57888	cytotoxic T-lymphocyte-associated serine esterase 1	CTLA1	14q11.2	Enzymes and other pathway molecules
31961_r_at	1.46	1.54	AF070579			3q27.1	Unclassified
38463_s_at	1.13	1.46	U29926	adenosine monophosphate deaminase (isoform E)	AMPD3	11p15	Enzymes and other pathway molecules
1538_s_at	1.36	4.36	X00695	interleukin 2	IL2	4q26-q27	Cytokines, chemokines and other ligands
34607_at	1.14	1.20	AB023135	inducible T-cell co-stimulator	ICOS	2q33	Cell surface molecule
40396_at	1.03	1.29	U49395	purinergic receptor P2X, ligand-gated ion channel, 5	P2RX5	17p13	Structural and intracellular rafficking molecules
1004_at	1.13	1.02	X68149	Burkitt lymphoma receptor 1, GTP-binding protein	BLR1	11q23.3	Cell surface molecule
441_s_at	1.05	1.01	X13967	leukemia inhibitory factor (cholinergic differentiation factor)	LIF	22q12.2	Cell surface molecule

Probe_ID	RL3_Signal Log Ratio	RI4_Signal Log Ratio	Accession	Gene name	Gene symbol	Locus	Functional group
34678_at	-1.01	-1.15	AL096713	fer-1-like 3, myoferlin	FER1L3	10q24	Unclassified
35712_at	-1.00	-1.40	AC004142	leucine-rich repeat protein, neuronal 3	LRRN3	7q22.3	Unclassified
34091_s_at	-1.44	-1.02	Z19554	vimentin	VIM	10p13	Structural and intracellular rafficking molecules
40456_at	-1.46	-1.00	AL049963				Unclassified
33541_s_at	-1.35	-1.17	AA133246	leukocyte-associated Ig-like receptor 2	LAIR2	19q13.4	Cell surface molecule
36899_at	-1.34	-1.25	M97287	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)	SATB1	3p23	Structural and intracellular rafficking molecules
36926_at	-1.59	-1.12	X80692	mitogen-activated protein kinase 6	MAPK6	15q21	Enzymes and other pathway molecules
649_s_at	-1.47	-1.44	L06797	chemokine (C-X-C motif), receptor 4 (fusin)	CXCR4	2q21	Cell surface molecule
38685_at	-1.81	-1.11	AL035306	hypothetical protein MGC14797	MGC14797	Xq26	Unclassified
34348_at	-1.98	-1.20	U78095	serine protease inhibitor, Kunitz type, 2	SPINT2	19q13.1	Enzymes and other pathway molecules
40049_at	-1.01	-2.18	X76104	death-associated protein kinase 1	DAPK1	9q34.1	Enzymes and other pathway molecules
37544_at	-1.69	-1.53	X64318	nuclear factor, interleukin 3 regulated	NFIL3	9q22	Transcriptional regulator
39331_at	-1.00	-2.37	X79535	tubulin, beta polypeptide	TUBB	6p21.3	Structural and intracellular rafficking molecules
1061_at	-1.51	-1.02	U00672	interleukin 10 receptor, alpha	IL10RA	11q23	Cell surface molecule
31856_at	-2.42	-1.25	Z24680	glycoprotein A repetitions predominant	GARP	11q13.5-q14	Cell surface molecule
931_at	-1.49	-2.36	L08177	Epstein-Barr virus induced gene 2 (lymphocyte-specific G protein-coupled receptor)	EBI2	13q32.3	Cell surface molecule
41504_s_at	-1.17	-2.86	AF055376	v-maf musculoaponeurotic fibrosarcoma (avian) oncogene homolog	MAF	16q22-q23	Transcriptional regulator
41193_at	-1.13	-2.93	AB013382	dual specificity phosphatase 6	DUSP6	12q22-q23	Enzymes and other pathway molecules
494_at	-1.85	-2.36	U31120	interleukin 13	IL-13	5q31	Cytokines, chemokines and other ligands
1069_at	-1.70	-2.80	U04636		Cox-2	cyclooxygenase-2	Enzymes and other pathway molecules

Probe_ID	RL3_Signal Log Ratio	RI4_Signal Log Ratio	Accession	Gene name	Gene symbol	Locus	Functional group
33809_at	-3.40	-1.19	AL049933	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1	GNAI1	7q21	Cell surface molecule
1062_g_at	-2.79	-1.98	U00672	interleukin 10 receptor, alpha	IL10RA	11q23	Cell surface molecule
37038_at	-2.33	-2.67	X83467	peroxisomal membrane protein-1	PXMP1	1p22-p21	Structural and intracellular rafficking molecules
41592_at	-3.68	-2.03	AB000734	JAK binding protein	SSI-1	16p13.13	Enzymes and other pathway molecules
36805_s_at	-2.85	-3.21	X03541	neurotrophic tyrosine kinase, receptor, type 1	NTRK1	1q21-q22	Cell surface molecule
1892_s_at	-3.26	-3.96	HG1437-HT1437	neurotrophic tyrosine kinase, receptor, type 1	Trk	1q21-q22	Cell surface molecule
40511_at	-3.68	-2.59	X58072	GATA-binding protein 3	GATA3	10p15	Transcriptional regulator
35938_at	-2.99	-3.80	M72393	phospholipase A2, group IVA (cytosolic, calcium-dependent)	PLA2G4A	1q25	Enzymes and other pathway molecules
41772_at	-4.00	-2.94	M68840	monoamine oxidase A	MAOA	Xp11.4-p11.3	Enzymes and other pathway molecules

TABLE 1 (continued)

Summary of data on genes differentially expressed at 48 h when gene expression of human CD4+ T cells induced to Th1 direction and treated with TGFb is compared to gene expression of human CD4+ T cells induced to Th1 direction.

Probe_ID	RI3_Signal Log Ratio	MAS5RL4_Signal Log Ratio	Accession	Gene name	Gene symbol	Locus	Functional group
1405_i_at	3.78	3.35	M21121	small inducible cytokine A5 (RANTES)	SCYA5	17q11.2-q12	Cytokines, chemokines and other ligands
1403_s_at	2.11	3.73	M21121	small inducible cytokine A5 (RANTES)	SCYA5	17q11.2-q12	Cytokines, chemokines and other ligands
40385_at	2.03	2.55	U64197	small inducible cytokine subfamily A (Cys-Cys), member 20	SCYA20	2q33-q37	Cytokines, chemokines and other ligands
34091_s_at	2.61	1.73	Z19554	vimentin	VIM	10p13	Structural and intracellular rafficking molecules
38391_at	1.14	3.04	M94345	capping protein (actin filament), gelsolin-like	CAPG	2cen-q24	Structural and intracellular rafficking molecules
41209_at	1.80	2.30	M15856	lipoprotein lipase	LPL	8p22	Enzymes and other pathway molecules
41472_at	1.76	2.15	AL078641	phorbolin-like protein MDS019	MDS019	22q	Unclassified
37669_s_at	1.62	2.27	U16799	ATPase, Na+/K+ transporting, beta 1 polypeptide	ATP1B1	1q22-q25	Structural and intracellular rafficking molecules
1788_s_at	2.34	1.49	U48807	dual specificity phosphatase 4	DUSP4	8p12-p11	Enzymes and other pathway molecules
34947_at	1.57	1.87	AA442560	phorbolin-like protein MDS019	MDS019	22q	Unclassified
32607_at	1.38	1.85	AF039656	brain abundant, membrane attached signal protein 1	BASP1	5p15.1-p14	Enzymes and other pathway molecules
31540_at	1.37	1.80	U03397	tumor necrosis factor receptor superfamily, member 9	TNFRSF9	1p36	Cell surface molecule
33232_at	1.53	1.33	AI017574	cysteine-rich protein 1 (intestinal)	CRIP1	7q11.23	Unclassified
40662_g_at	1.00	1.70	D78579	nuclear receptor subfamily 4, group A, member 3	NR4A3	9q22	Unclassified
2069_s_at	1.18	1.46	L23805	catenin (cadherin-associated protein), alpha 1 (102Kd)	CTNNA1	5q31	Cell surface molecule
41779_at	1.13	1.51	U70426	regulator of G-protein rafficki 16	RGS16	1q25-q31	Cell surface molecule

Probe_ID	RI3_Signal Log Ratio	MAS5RL4_Signal Log Ratio	Accession	Gene name	Gene symbol	Locus	Functional group
769_s_at	1.28	1.28	D00017	annexin A2	ANXA2	15q21-q22	Enzymes and other pathway molecules
1358_s_at	1.02	1.27	U22970	interferon, alpha-inducible protein	G1P3	1p35	Unclassified
33412_at	1.01	1.25	AI535946	lectin, galactoside-binding, soluble, 1 (galectin 1)	LGALS1	22q13.1	Enzymes and other pathway molecules
31444_s_at	1.10	1.08	M62895	annexin A2 pseudogene 3	ANXA2P3	10q21-q22	Unclassified
40365_at	-1.09	-1.08	M63904	guanine nucleotide binding protein (G protein), alpha 15 (Gq class)	GNA15	19p13.3	Cell surface molecule
36545_s_at	-1.12	-1.15	AB011114	KIAA0542 gene product	KIAA0542	22q12.2	Unclassified
37137_at	-1.53	-1.24	M17016	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	GZMB	14q11.2	Enzymes and other pathway molecules
37544_at	-1.75	-1.68	X64318	nuclear factor, interleukin 3 regulated	NFIL3	9q22	Transcriptional regulator
33285_i_at	-2.54	-1.00	W26762	hypothetical protein FLJ21168	FLJ21168	1p11.1	Unclassified
36899_at	-1.82	-1.78	M97287	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)	SATB1	3p23	Structural and intracellular rafficking molecules
38944_at	-2.55	-3.13	U68019	MAD (mothers against decapentaplegic, Drosophila) homolog 3	MADH3	15q21-q22	Transcriptional regulator

TABLE 1 (continued)

Summary of data on genes differentially expressed at 48 h when gene expression of human CD4+ T cells induced to Th2 direction and treated with TGFb is compared to gene expression of human CD4+ T cells induced to Th2 direction.

Probe_ID	RI3_Signal Log Ratio	RL4_Signal Log Ratio	Accession	Gene name	Gene symbol	Locus	Functional group
37669_s_at	3.04	4.00	U16799	ATPase, Na+/K+ transporting, beta 1 polypeptide	ATP1B1	1q22-q25	Structural and intracellular trafficing molecules
1183_at	1.83	4.84	D43767	small inducible cytokine subfamily A (Cys-Cys), member 17	SCYA17	16q13	Cytokines, chemokines and other ligands
1405_i_at	2.26	3.91	M21121	small inducible cytokine A5 (RANTES)	SCYA5	17q11.2-q12	Cytokines, chemokines and other ligands
977_s_at	3.75	2.28	Z35402	cadherin 1, type 1, E-cadherin	ECAD3	16q22.1	Cell surface molecule
35367_at	1.98	3.13	AB006780	lectin, galactoside-binding, soluble, 3 (galectin 3)	LGALS3	14q21-q22	Enzymes and other pathway molecules
1403_s_at	2.01	2.87	M21121	small inducible cytokine A5 (RANTES)	SCYA5	17q11.2-q12	Cytokines, chemokines and other ligands
32607_at	2.09	2.01	AF039656	brain abundant, membrane attached signal protein 1	BASP1	5p15.1-p14	Enzymes and other pathway molecules
34037_at	1.91	1.98	M30134	interleukin 9	IL9	5q31.1	Cytokines, chemokines and other ligands
35824_at	1.74	2.04	AJ223321	zinc finger protein 238	ZNF238	1q44-qter	Transcriptional regulator
36575_at	2.06	1.70	S59049	regulator of G-protein signalling 1	RGS1	1q31	Cell surface molecule
36117_at	2.17	1.46	L13616	PTK2 protein tyrosine kinase 2	PTK2	8q24-qter	Enzymes and other pathway molecules
34739_at	1.37	2.20	W26023	hypothetical protein FLJ20275	FLJ20275	1p21.3	Unclassified
37603_at	1.42	1.98	X52015	interleukin 1 receptor antagonist	IL1RN	2q14.2	Cytokines, chemokines and other ligands
39248_at	1.85	1.45	N74607	aquaporin 3	AQP3	9p13	Structural and intracellular trafficing molecules
34217_at	1.08	1.52	AB015132	Kruppel-like factor 7 (ubiquitous)	KLF7	2q32	Transcriptional regulator
37377_i_at	1.22	1.35	M13452	lamin A/C	LMNA	1q21.2-q21.3	Structural and intracellular trafficing molecules
40818_at	1.15	1.35	D14041	H-2K binding factor-2	LOC51580	9	Transcriptional regulator
37391_at	1.07	1.32	X12451	cathepsin L	CTSL	9q21-q22	Unclassified
32587_at	1.18	1.14	U07802	zinc finger protein 36, C3H type-like 2	ZFP36L2	2p22.3-p21	Transcriptional regulator
32778_at	-1.10	-1.06	D26070	inositol 1,4,5-triphosphate receptor, type 1	ITPR1	3p26-p25	Structural and intracellular

Probe_ID	RI3_Signal Log Ratio	RL4_Signal Log Ratio	Accession	Gene name	Gene symbol	Locus	Functional group
							trafficing molecules
38006_at	-1.15	-1.06	M37766	CD48 antigen (B-cell membrane protein)	CD48	1q21.3-q22	Cell surface molecule
41215_s_at	-1.11	-1.14	D13891	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	ID2	2p25	Enzymes and other pathway molecules
33849_at	-1.06	-1.35	U02020	pre-B-cell colony-enhancing factor	PBEF	7q22.1	Cytokines, chemokines and other ligands
34801_at	-1.33	-1.25	AB014610	KIAA0710 gene product	KIAA0710	12q13.2-q13.3	Unclassified
34098_f_at	-1.38	-1.22	AI799757	integrin cytoplasmic domain-associated protein 1	ICAP-1A	2p25.2	Cell surface molecule
36377_at	-1.44	-1.18	U43672	interleukin 18 receptor 1	IL18R1	2q12	Cell surface molecule
1069_at	-1.12	-1.51	U04636	cyclooxygenase-2	Cox-2	1q25.2-q25.3	Enzymes and other pathway molecules
38122_at	-1.49	-1.23	D87075	solute carrier family 23 (nucleobase transporters), member 1	SLC23A1	20p13	Structural and intracellular trafficing molecules
35943_s_at	-1.13	-1.88	D13317	GA-binding protein transcription factor, beta subunit 1 (53kD)	GABPB1	7q11.2	Transcriptional regulator
2002_s_at	-1.29	-1.75	U27467	BCL2-related protein A1	BCL2A1	15q24.3	Cytokines, chemokines and other ligands
33809_at	-1.15	-1.97	AL049933	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1	GNAI1	7q21	Cell surface molecule
34965_at	-1.54	-1.59	AF031824	cystatin F (leukocystatin)	CST7	20p11.22-p11.21	Enzymes and other pathway molecules
37544_at	-1.23	-1.95	X64318	nuclear factor, interleukin 3 regulated	NFIL3	9q22	Transcriptional regulator
36160_s_at	-1.27	-2.04	U81561	protein tyrosine phosphatase, receptor type, N polypeptide 2	PTPRN2	7q36	Enzymes and other pathway molecules
35938_at	-1.15	-2.86	M72393	phospholipase A2, group IVA (cytosolic, calcium-dependent)	PLA2G4A	1q25	Enzymes and other pathway molecules

Table 2

Novel target genes of IL-12 in human CD4+ T cells (Th1-induced vs activated)

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
2h	32417_at	desmocollin 3	**DSC3**	D17427	**2.8**
	39718_r_at	mitochondrial ribosomal protein L33	**MRPL33**	N98607	**-4.0**
48h	40635_at	flotillin 1	**FLOT1**	AF089750	**2.5**
	34654_at	myotubularin related protein 1	**MTMR1**	AJ224979	**2.1**
	38620_at	golgi SNAP receptor complex member 2	**GOSR2**	AA905543	**-2.3**
	34866_at		**.**	AF055029	**-2.8**
	31856_at	glycoprotein A repetitions predominant	**GARP**	Z24680	**-2.9**
	39582_at		**.**	AL050166	**-2.9**
	38575_at		**MALT1**	AF070528	**-3.0**
	33541_s_at	leukocyte-associated Ig-like receptor 2	**LAIR2**	AA133246	**-3.4**

TABLE 2 (continued)

Novel target genes of IL-4 in human CD4+ T cells (Th2-induced vs activated))

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
2h	38549_at	Vipirin	**cig5**	AF026941	**13.5**
	34757_at	ADP-ribosyltransferase (NAD+; poly(ADP-ribose) polymerase)-like 2	**ADPRTL2**	AA595596	**4.6**
	41193_at	dual specificity phosphatase 6	**DUSP6**	AB013382	**4.0**
	39593_at	Homo sapiens, Similar to fibrinogen-like 2, clone MGC:22391 IMAGE:4616866, mRNA, complete cds	.	AI432401	**3.9**
	38149_at	KIAA0053 gene product	**KIAA0053**	D29642	**3.3**
	35712_at	leucine-rich repeat protein, neuronal 3	**LRRN3**	AC004142	**3.0**
	33047_at	ESTs, Weakly similar to B34087 hypothetical protein [H.sapiens]	.	AI971169	**2.9**
	38051_at	mal, T-cell differentiation protein	**MAL**	X76220	**2.7**
	36736_f_at	phosphoserine phosphatase	**PSPH**	Y10275	**2.5**
	35320_at	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2	**SLC11A2**	AB004857	**2.3**
	36899_at	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)	**SATB1**	M97287	**2.3**
	725_i_at	Chorionic Somatomammotropin Hormone Cs-5		HG1751-HT1768	**2.3**
	41145_at	KIAA0914 gene product	**KIAA0914**	AB020721	**2.1**
	39827_at	HIF-1 responsive RTP801	**RTP801**	AA522530	**-2.1**
	41475_at	ninjurin 1	**NINJ1**	U91512	**-2.3**
	33997_at	Homo sapiens mRNA; cDNA DKFZp586B1722 (from clone DKFZp586B1722)	.	AL049449	**-2.8**
	37043_at	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	**ID3**	AL021154	**-2.8**
6h	41193_at	dual specificity phosphatase 6	**DUSP6**	AB013382	**8.6**
	38549_at	Vipirin	**cig5**	AF026941	**7.7**
	34348_at	serine protease inhibitor, Kunitz type, 2	**SPINT2**	U78095	**6.7**
	39330_s_at	actinin, alpha 1	**ACTN1**	M95178	**3.9**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	34173_s_at	contactin 5	**CNTN5**	AB013802	**3.4**
	34319_at	S100 calcium binding protein P	**S100P**	AA131149	**3.1**
	37038_at	ATP-binding cassette, sub-family D (ALD), member 3	**ABCD3**	X83467	**3.0**
	40839_at	ubiquitin-like 3	**UBL3**	AL080177	**2.7**
	33352_at	H2B histone family, member Q	**H2BFQ**	X57985	**2.6**
	41577_at	protein phosphatase 1, regulatory (inhibitor) subunit 16B	**PPP1R16B**	AB020630	**2.5**
	38051_at	mal, T-cell differentiation protein	**MAL**	X76220	**2.2**
	34256_at	sialyltransferase 9 (CMP-NeuAc:lactosylceramide alpha-2,3-sialyltransferase; GM3 synthase)	**SIAT9**	AB018356	**-2.1**
	41209_at	lipoprotein lipase	**LPL**	M15856	**-2.3**
	41744_at	Optineurin	**OPTN**	AF070533	**-2.6**
	32649_at	transcription factor 7 (T-cell specific, HMG-box)	**TCF7**	X59871	**-2.6**
	36239_at	POU domain, class 2, associating factor 1	**POU2AF1**	Z49194	**-2.9**
	37043_at	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	**ID3**	AL021154	**-4.4**
	33540_at	Homo sapiens mRNA; cDNA DKFZp564A023 (from clone DKFZp564A023)		AL049233	**-4.6**
	39586_at	Desmoglein 1	**DSG1**	AF097935	**-4.6**
48h	41193_at	dual specificity phosphatase 6	**DUSP6**	AB013382	**8.7**
	34439_at	absent in melanoma 2	**AIM2**	AF024714	**7.9**
	37038_at	peroxisomal membrane protein-1	**ABCD3**	X83467	**5.9**
	34678_at	fer-1-like 3, myoferlin	**FER1L3**	AL096713	**3.7**
	36899_at	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)	**SATB1**	M97287	**3.4**
	38336_at	KIAA1013 protein	**KIAA1013**	AB023230	**3.2**
	41215_s_at	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	**ID2**	D13891	**2.7**
	40848_g_at	KIAA0750 gene product	**KIAA0750**	AB018293	**2.5**
	37524_at	serine/threonine kinase 17b (apoptosis-inducing)	**STK17B**	AB011421	**2.4**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	34517_at	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	**HMGCS1**	X66435	**2.4**
	34348_at	serine protease inhibitor, Kunitz type, 2	**SPINT2**	U78095	**2.3**
	40448_at	zinc finger protein 36, C3H type, homolog (mouse)	**ZFP36**	M92843	**2.2**
	37732_at		**RYBP**	AL049940	**2.2**
	37616_at	AU RNA-binding protein/enoyl-Coenzyme A hydratase	**AUH**	X79888	**2.2**
	37842_at	I-mfa domain-containing protein	.	AF054589	**-2.0**
	33339_g_at	CD83 antigen (activated B lymphocytes, immunoglobulin superfamily)	**CD83**	Z11697	**-2.2**
	38575_at		**MALT1**	AF070528	**-3.0**

TABLE 2 (continued)

Novel data on genes differentially expressed at 48 h when gene expression of human CD4+ T cells induced to Th1 direction is compared to gene expression of human CD4+ T cells induced to Th2 direction.

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
2h	37043_at	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	**ID3**	AL021154	**2.6**
	37909_at	laminin, alpha 3 (nicein (150kD), kalinin (165kD), BM600 (150kD), epilegrin)	**LAMA3**	L34155	**2.4**
	36160_s_at	protein tyrosine phosphatase, receptor type, N polypeptide 2	**PTPRN2**	U81561	**2.3**
	39827_at	HIF-1 responsive RTP801	**RTP801**	AA522530	**2.1**
	41475_at	ninjurin 1	**NINJ1**	U91512	**2.0**
	35320_at	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2	**SLC11A2**	AB004857	**-2.1**
	37524_at	serine/threonine kinase 17b (apoptosis-inducing)	**STK17B**	AB011421	**-2.2**
	32919_at	Homo sapiens, clone IMAGE:3625286, mRNA, partial cds	.	AC004010	**-2.3**
	39549_at	hypothetical protein FLJ23138	**FLJ23138**	AI743090	**-2.3**
	33352_at	H2B histone family, member Q	**H2BFQ**	X57985	**-2.4**
	36899_at	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)	**SATB1**	M97287	**-2.5**
	38051_at	mal, T-cell differentiation protein	**MAL**	X76220	**-2.6**
	34348_at	serine protease inhibitor, Kunitz type, 2	**SPINT2**	U78095	**-2.6**
	35712_at	leucine-rich repeat protein, neuronal 3	**LRRN3**	AC004142	**-2.6**
	38149_at	KIAA0053 gene product	**KIAA0053**	D29642	**-2.9**
	36435_at	protein phosphatase 1A (formerly 2C), magnesium-dependent, alpha isoform	**PPM1A**	AF070670	**-3.0**
	32058_at	HNK-1 sulfotransferase	**HNK-1ST**	AF070594	**-3.0**
	36148_at	amyloid beta (A4) precursor-like protein 1	**APLP1**	U48437	**-3.5**
	41193_at	dual specificity phosphatase 6	**DUSP6**	AB013382	**-3.7**
	33047_at	ESTs, Weakly similar to B34087 hypothetical protein	.	AI971169	**-4.0**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
		[H.sapiens]			
	32148_at	FERM, RhoGEF (ARHGEF) and pleckstrin domain protein 1 (chondrocyte-derived)	**FARP1**	AI701049	**-4.3**
	39593_at	Homo sapiens, Similar to fibrinogen-like 2, clone MGC:22391 IMAGE:4616866, mRNA, complete cds	.	AI432401	**-9.2**
	38549_at	Vipirin	**cig5**	AF026941	**-35.5**
6h	40757_at	granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)	**GZMA**	M18737	**3.6**
	32041_r_at	CDC5 cell division cycle 5-like (S. pombe)	**CDC5L**	AB007892	**3.5**
	36705_at	protein kinase, AMP-activated, beta 2 non-catalytic subunit	**PRKAB2**	AJ224538	**3.1**
	32649_at	transcription factor 7 (T-cell specific, HMG-box)	**TCF7**	X59871	**2.9**
	36239_at	POU domain, class 2, associating factor 1	**POU2AF1**	Z49194	**2.7**
	34252_at	hypothetical protein FLJ10342	**FLJ10342**	W28545	**2.6**
	37485_at	fatty-acid-Coenzyme A ligase, very long-chain 1	**FACVL1**	D88308	**2.2**
	40698_at	C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 2 (activation-induced)	**CLECSF2**	X96719	**2.2**
	34256_at	sialyltransferase 9 (CMP-NeuAc:lactosylceramide alpha-2,3-sialyltransferase; GM3 synthase)	**SIAT9**	AB018356	**2.0**
	37025_at	LPS-induced TNF-alpha factor	**PIG7**	AL120815	**2.0**
	38051_at	mal, T-cell differentiation protein	**MAL**	X76220	**-2.1**
	34319_at	S100 calcium binding protein P	**S100P**	AA131149	**-2.2**
	37038_at	ATP-binding cassette, sub-family D (ALD), member 3	**ABCD3**	X83467	**-2.4**
	40839_at	ubiquitin-like 3	**UBL3**	AL080177	**-2.4**
	35794_at	KIAA0942 protein	**KIAA0942**	AB023159	**-2.5**
	39330_s_at	actinin, alpha 1	**ACTN1**	M95178	**-2.6**
	35422_at	microtubule-associated protein 2	**MAP2**	U01828	**-3.6**
	34990_at	SET binding protein 1	**SETBP1**	AB022660	**-4.4**
	34348_at	serine protease inhibitor, Kunitz type, 2	**SPINT2**	U78095	**-5.9**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	38549_at	Vipirin	**cig5**	AF026941	**-6.1**
	41193_at	dual specificity phosphatase 6	**DUSP6**	AB013382	**-6.7**
48h	39264_at	2'-5'-oligoadenylate synthetase 2 (69-71 kD)	**OAS2**	M87284	**4.0**
	31961_r_at		**.**	AF070579	**2.8**
	34678_at	fer-1-like 3, myoferlin	**FER1L3**	AL096713	**-2.1**
	35712_at	leucine-rich repeat protein, neuronal 3	**LRRN3**	AC004142	**-2.3**
	33541_s_at	leukocyte-associated Ig-like receptor 2	**LAIR2**	AA133246	**-2.4**
	36899_at	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)	**SATB1**	M97287	**-2.5**
	38685_at	hypothetical protein MGC14797	**MGC14797**	AL035306	**-2.8**
	34348_at	serine protease inhibitor, Kunitz type, 2	**SPINT2**	U78095	**-3.0**
	40049_at	death-associated protein kinase 1	**DAPK1**	X76104	**-3.0**
	31856_at	glycoprotein A repetitions predominant	**GARP**	Z24680	**-3.6**
	41193_at	dual specificity phosphatase 6	**DUSP6**	AB013382	**-4.1**
	37038_at	peroxisomal membrane protein-1	**ABCD3**	X83467	**-5.7**

Table 3

Target genes of IL-4 in murine CD4+ T cells (Th2-induced vs activated)

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
2h	103841_at	zinc finger protein 64	**Zfp64**	U49046	**-3.5**
	96623_at	UDP-glucose ceramide glucosyltransferase-like	**Ugcgl**	AI853172	**-2.3**
	102281_at	tumor necrosis factor receptor superfamily, member 7	**Tnfrsf7**	L24495	**-2.4**
	160273_at	zinc finger protein 36, C3H type-like 2	**Zfp36l2**	AA960603	**-2.3**
	92368_at	receptor (calcitonin) activity modifying protein 3	**Ramp3**	AJ250491	**-2.2**
	98478_at	cyclin G2	**Ccng2**	U95826	**-2.1**
	98072_r_at	deoxycytidine kinase	**Dck**	X77731	**-2.0**
	104371_at	diacylglycerol O-acyltransferase 1	**Dgat1**	AF078752	**-1.9**
	102797_at	retinal short-chain dehydrogenase/reductase 1	**Rsdr1-pending**	X95281	**2.0**
	160439_at	polymerase, gamma	**Polg**	U53584	**2.0**
	96515_at	interleukin-four induced gene 1	**Fig1**	U70430	**1.9**
	104400_at	prenylated SNARE protein	**Ykt6-pending**	AF076956	**2.3**
	100606_at	prion protein	**Prnp**	M18070	**2.3**
	104449_at	glycine receptor, beta subunit	**Glrb**	X81202	**2.2**
	99021_at	paired related homeobox 1	**Prrx1**	U03873	**2.5**
	102021_at	interleukin 4 receptor, alpha	**Il4ra**	M27960	**2.5**
	104155_f_at	activating transcription factor 3	**Atf3**	U19118	**2.5**
	100022_at	cytokine inducible SH2-containing protein	**Cish**	D89613	**2.5**
	99917_at	enhancer of zeste homolog 2 (Drosophila)	**Ezh2**	U52951	**2.5**
	97523_i_at	amylase 2, pancreatic	**Amy2**	X02578	**2.6**
	104156_r_at	activating transcription factor 3	**Atf3**	U19118	**2.8**
	160948_at	protein phosphatase 3, catalytic subunit, gamma isoform	**Ppp3cc**	M81475	**2.8**
	98869_g_at	B-cell leukemia/lymphoma 2	**Bcl2**	L31532	**3.5**
	98868_at	B-cell leukemia/lymphoma 2	**Bcl2**	L31532	**3.7**
	161411_i_at	lipase, hormone sensitive	**Lipe**	AV315398	**8.1**
	161491_r_at	f-box only protein 3	**Fbxo3**	AV083174	**10.5**
	101920_at	DNA polymerase epsilon, subunit 2	**Pole2**	AF036898	**15.1**
6h	100131_at	secretory granule neuroendocrine protein 1, 7B2 protein	**Sgne1**	X15830	**-6.0**
	102157_f_at	immunoglobulin kappa chain variable 28 (V28)	**Igk-V28**	M15520	**-4.7**
	161997_f_at	aldehyde dehydrogenase 2, mitochondrial	**Aldh2**	AV329607	**-2.9**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	92280_at	actinin, alpha 1	**Actn1**	AA867778	**-2.2**
	92608_at	cysteine rich protein	**Csrp**	D88793	**-2.1**
	101965_at	ring finger protein 13	**Rnf13**	AF037205	**-2.0**
	92614_at	inhibitor of DNA binding 3	**Idb3**	M60523	**-2.0**
	100022_at	cytokine inducible SH2-containing protein	**Cish**	D89613	**2.0**
	96515_at	interleukin-four induced gene 1	**Fig1**	U70430	**2.0**
	98869_g_at	B-cell leukemia/lymphoma 2	**Bcl2**	L31532	**2.0**
	100924_at	GATA binding protein 3	**Gata3**	X55123	**2.2**
	104156_r_at	activating transcription factor 3	**Atf3**	U19118	**2.2**
	102021_at	interleukin 4 receptor, alpha	**Il4ra**	M27960	**2.3**
	104155_f_at	activating transcription factor 3	**Atf3**	U19118	**2.4**
	98868_at	B-cell leukemia/lymphoma 2	**Bcl2**	L31532	**2.5**
	101913_at	hairy/enhancer-of-split related with YRPW motif 1	**Hey1**	AW214298	**2.6**
	161051_at	hairy and enhancer of split 5, (Drosophila)	**Hes5**	D32132	**2.6**
	160886_i_at	fatty acid binding protein 6, ileal (gastrotropin)	**Fabp6**	AV063979	**3.9**
	100127_at	cellular retinoic acid binding protein II	**Crabp2**	M35523	**5.1**
	104728_at	protein S (alpha)	**Pros1**	L27439	**5.7**
24h	160726_at	Quaking	**qk**	U44940	**-11.4**
	161293_r_at	proteasome (prosome, macropain) 28 subunit, alpha	**Psme1**	AV306568	**-10.1**
	94774_at	interferon activated gene 202A	**Ifi202a**	M31418	**-2.9**
	102424_at	small inducible cytokine A3	**Scya3**	J04491	**-2.6**
	99334_at	interferon gamma	**Ifng**	K00083	**-2.6**
	161609_at	regulator of G-protein signaling 16	**Rgs16**	AV349152	**-2.5**
	94224_s_at	interferon activated gene 205	**Ifi205**	M74123	**-2.3**
	96060_at	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6	**Serpinb6**	U25844	**-2.3**
	96592_at	phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)	**Pik3r1**	U50413	**-2.2**
	101851_at	antigen identified by monoclonal antibody MRC OX-2	**Mox2**	AF029215	**-2.1**
	93321_at	interferon activated gene 203	**Ifi203**	AF022371	**-2.1**
	103279_at	SH2 domain protein 1A	**Sh2d1a**	AF097632	**-2.1**
	161948_f_at	myosin Va	**Myo5a**	AV214912	**-2.0**
	102630_s_at	lymphotoxin A	**Lta**	M16819	**-2.0**
	94146_at	small inducible cytokine A4	**Scya4**	X62502	**-2.0**
	102921_s_at	tumor necrosis factor receptor superfamily,	**Tnfrsf6**	M83649	**-2.0**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
		member 6			
	102638_at	cystatin F (leukocystatin)	**Cst7**	AB015224	**2.0**
	93871_at	interleukin 1 receptor antagonist	**Il1rn**	L32838	**2.0**
	92234_at	retinoid X receptor alpha	**Rxra**	X66223	**2.0**
	98282_at	inducible T-cell co-stimulator	**Icos**	AB023132	**2.0**
	93092_at	histocompatibility 2, class II, locus DMa	**H2-DMa**	U35323	**2.1**
	161012_at	immunoglobulin-associated beta	**Igb**	J03857	**2.1**
	AFFX-MurIL4_at	interleukin 4	**Il4**	M25892	**2.1**
	92286_g_at	interleukin 4	**Il4**	AA967539	**2.1**
	99895_at	chemokine (C-C) receptor 8	**Cmkbr8**	AF001277	**2.2**
	96515_at	interleukin-four induced gene 1	**Fig1**	U70430	**2.2**
	102995_s_at	granzyme A	**Gzma**	M13226	**2.3**
	93444_at	basic leucine zipper transcription factor, ATF-like	**Batf**	AF017021	**2.3**
	101902_at	recombining binding protein suppressor of hairless (Drosophila)	**Rbpsuh**	X17459	**2.5**
	102021_at	interleukin 4 receptor, alpha	**Il4ra**	M27960	**3.2**
	93750_at	Gelsolin	**Gsn**	J04953	**3.9**
	100924_at	GATA binding protein 3	**Gata3**	X55123	**4.0**
	103833_at	homeodomain interacting protein kinase 2	**Hipk2**	AF077659	**4.2**
	94458_at	caspase 6	**Casp6**	Y13087	**5.2**
	92382_at	myosin VI	**Myo6**	U49739	**5.3**
	104728_at	protein S (alpha)	**Pros1**	L27439	**6.4**
48h	93321_at	interferon activated gene 203	**Ifi203**	AF022371	**-6.8**
	104669_at	interferon regulatory factor 7	**Irf7**	U73037	**-5.5**
	103432_at	interferon-stimulated protein (20 kDa)	**Isg20**	AW122677	**-5.4**
	93880_at	eomesodermin homolog (Xenopus laevis)	**Eomes**	AB031037	**-5.1**
	100981_at	interferon-induced protein with tetratricopeptide repeats 1	**Ifit1**	U43084	**-4.9**
	94224_s_at	interferon activated gene 205	**Ifi205**	M74123	**-4.9**
	97113_at	tumor necrosis factor (ligand) superfamily, member 6	**Tnfsf6**	U06948	**-4.4**
	103554_at	a disintegrin and metalloproteinase domain 19 (meltrin beta)	**Adam19**	AA726223	**-4.3**
	160878_at	block of proliferation 1	**Bop1**	AF061503	**-4.2**
	96060_at	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6	**Serpinb6**	U25844	**-4.2**
	99334_at	interferon gamma	**Ifng**	K00083	**-3.9**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	102906_at	T-cell specific GTPase	**Tgtp**	L38444	**-3.8**
	101144_at	interleukin 18 receptor 1	**Il18r1**	U43673	**-3.6**
	99461_at	hematopoietic cell specific Lyn substrate 1	**Hcls1**	X84797	**-3.5**
	98446_s_at	Eph receptor B4	**Ephb4**	U06834	**-3.4**
	98465_f_at	interferon activated gene 204	**Ifi204**	M31419	**-3.4**
	102635_at	vesicle transport through interaction with t-SNAREs 1 homolog	**Vti1-pending**	AF035823	**-3.3**
	96764_at	interferon-inducible GTPase	**Igp-pending**	AJ007971	**-3.2**
	94855_at	Prohibitin	**Phb**	X78682	**-3.2**
	98822_at	interferon-stimulated protein (15 kDa)	**Isg15**	X56602	**-3.2**
	104093_at	lymphocyte specific 1	**Lsp1**	D49691	**-3.1**
	101571_g_at	insulin-like growth factor binding protein 4	**Igfbp4**	X76066	**-3.1**
	103367_at	UDP-N-acetyl-alpha-D-galactosamine:(N-acetylneuraminyl)-galactosylglucosylceramide-beta-1, 4-N-acetylgalactosaminyltransferase	**Galgt1**	U18975	**-2.9**
	96046_at	histone deacetylase 1	**Hdac1**	X98207	**-2.9**
	93290_at	purine-nucleoside phosphorylase	**Pnp**	U35374	**-2.9**
	160503_at	Fibrillarin	**Fbl**	Z22593	**-2.9**
	97507_at	peptidylprolyl isomerase C-associated protein	**Ppicap**	X67809	**-2.9**
	96297_at	EBNA1 binding protein 2	**Ebp2**	AI845934	**-2.8**
	93218_at	SWAP complex protein, 70 kDa	**Swap70**	AF053974	**-2.8**
	92220_s_at	myc box dependent interacting protein 1	**Bin1**	U60884	**-2.8**
	93924_f_at	tubulin, alpha 7	**Tuba7**	M13443	**-2.7**
	98589_at	adipose differentiation related protein	**Adfp**	M93275	**-2.6**
	100154_at	TAP binding protein	**Tapbp**	AI836367	**-2.6**
	97875_at	adhesion regulating molecule 1	**Adrm1**	AW123694	**-2.6**
	93518_at	ribonucleic acid binding protein S1	**Rnps1**	X70067	**-2.5**
	160944_at	plakophilin 3	**Pkp3**	AW120906	**-2.4**
	95733_at	solute carrier family 29 (nucleoside transporters), member 1	**Slc29a1**	AI838274	**-2.4**
	92879_at	protein phosphatase 1G (formerly 2C), magnesium-dependent, gamma isoform	**Ppm1g**	U42383	**-2.4**
	161585_at	glycoprotein 5 (platelet)	**Gp5**	AV318587	**-2.4**
	103823_at	topoisomerase (DNA) III beta	**Tqp3b**	AB013603	**-2.4**
	160318_at	STIP1 homology and U-Box containing protein 1	**Stub1**	AI844511	**-2.4**
	99486_at	centromere autoantigen B	**Cenpb**	X55038	**-2.4**
	94815_at	2,3-bisphosphoglycerate mutase	**Bpgm**	X13586	**-2.4**

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TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	97251_at	mitochondrial ribosomal protein S10	**Mrps10**	AI842124	**-2.4**
	101486_at	proteasome (prosome, macropain) subunit, beta type 10	**Psmb10**	Y10875	**-2.4**
	104275_g_at	transformation related protein 53	**Trp53**	AB021961	**-2.4**
	100632_at	protein kinase, AMP-activated, gamma 1 non-catalytic subunit	**Prkag1**	AF036535	**-2.3**
	94168_at	interleukin 13	**Il13**	M23504	**-2.3**
	104025_at	thimet oligopeptidase 1	**Thop1**	AW047185	**-2.3**
	160106_at	capping protein (actin filament), gelsolin-like	**Capg**	X54511	**-2.3**
	99639_at	ubiquintin c-terminal hydrolase related polypeptide	**Uchrp**	D84096	**-2.3**
	92204_at	CD6 antigen	**Cd6**	U12434	**-2.3**
	92637_at	phosphofructokinase, liver, B-type	**Pfkl**	J03928	**-2.3**
	93364_at	Catenin alpha 1	**Catna1**	X59990	**-2.2**
	93892_at	CUG triplet repeat,RNA binding protein 2	**Cugbp2**	Y18298	**-2.2**
	103025_at	Moloney leukemia virus 10	**Mov10**	X52574	**-2.2**
	100962_at	Ngfi-A binding protein 2	**Nab2**	U47543	**-2.2**
	99669_at	lectin, galactose binding, soluble 1	**Lgals1**	X15986	**-2.2**
	104653_at	core binding factor beta	**Cbfb**	AA864065	**-2.2**
	101989_at	ubiquinol-cytochrome c reductase core protein 1	**Uqcrc1**	AW125380	**-2.1**
	97532_at	Protein phosphatase 5, catalytic subunit	**Ppp5c**	AF018262	**-2.1**
	98030_at	tripartite motif protein 30	**Trim30**	J03776	**-2.1**
	98339_at	synaptotagmin 11	**Syt11**	AB026808	**-2.1**
	100101_at	small nuclear ribonucleoprotein polypeptide A	**Snrpa**	L15447	**-2.1**
	101510_at	proteasome (prosome, macropain) 28 subunit, alpha	**Psme1**	AB007136	**-2.1**
	92540_f_at	spermidine synthase	**Srm**	Z67748	**-2.1**
	98153_at	chaperonin subunit 3 (gamma)	**Cct3**	L20509	**-2.1**
	102877_at	granzyme B	**Gzmb**	M12302	**-2.1**
	160290_at	insulin degrading enzyme	**Ide**	AI574278	**-2.1**
	99323_at	interleukin 12 receptor, beta 2	**Il12rb2**	U64199	**-2.1**
	97867_at	hydroxysteroid 11-beta dehydrogenase 1	**Hsd11b1**	X83202	**-2.1**
	92688_at	acid phosphatase 2, lysosomal	**Acp2**	X57199	**-2.1**
	104712_at	myelocytomatosis oncogene	**Myc**	L00039	**-2.0**
	160521_at	Nucleolin	**Ncl**	U01139	**-2.0**
	102791_at	proteosome (prosome, macropain) subunit, beta type 8 (large multifunctional protease 7)	**Psmb8**	U22033	**-2.0**
	99169_at	coactivator-associated arginine methyltransferase	**Carm1-pending**	AW122165	**-2.0**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
		1			
	101492_at	protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting 1	**Pin1**	AW047032	**-2.0**
	96883_at	eukaryotic translation initiation factor 3, subunit 4 (delta, 44 kDa)	**Eif3s4**	U70733	**-2.0**
	102257_at	Pbx/knotted 1 homeobox	**Pknox1**	AF061270	**-2.0**
	93500_at	aminolevulinic acid synthase 1	**Alas1**	M63245	**-2.0**
	103683_at	dihydroorotate dehydrogenase	**Dhodh**	AF029667	**-2.0**
	99478_at	protein tyrosine phosphatase, receptor type, C polypeptide-associated protein	**Ptprcap**	X97268	**-2.0**
	103946_at	proline-serine-threonine phosphatase-interacting protein 1	**Pstpip1**	U87814	**-2.0**
	102940_at	lymphotoxin B	**Ltb**	U16985	**-2.0**
	104137_at	ATP-binding cassette, sub-family A (ABC1), member 2	**Abca2**	X75927	**-2.0**
	100156_at	mini chromosome maintenance deficient 5 (S. cerevisiae)	**Mcmd5**	D26090	**-2.0**
	104102_at	protease, serine, 25	**Prss25**	AW047978	**-2.0**
	94138_s_at	Fucosyltransferase 7	**Fut7**	U45980	**-2.0**
	102778_at	immunoglobulin-associated alpha	**Iga**	X13450	**-2.0**
	92203_s_at	CD6 antigen	**Cd6**	U37543	**-1.9**
	94850_at	acyl-Coenzyme A thioesterase 3, mitochondrial	**Acate3-pending**	AJ238894	**-1.9**
	98500_at	interleukin 1 receptor-like 1	**Il1rl1**	D13695	**2.0**
	160439_at	polymerase, gamma	**Polg**	U53584	**2.0**
	103518_at	cytotoxic T lymphocyte-associated protein 2 beta	**Ctla2b**	X15592	**2.0**
	103259_at	Growth factor independent 1	**Gfi1**	U58972	**2.0**
	100606_at	prion protein	**Prnp**	M18070	**2.0**
	99143_at	trans-golgi network protein 2	**Tgoln2**	AA614914	**2.2**
	96912_s_at	cytotoxic T lymphocyte-associated protein 2 alpha	**Ctla2a**	X15591	**2.2**
	162172_f_at	neural precursor cell expressed, developmentally down-regulated gene 4a	**Nedd4a**	AV365271	**2.2**
	102955_at	nuclear factor, interleukin 3, regulated	**Nfil3**	U83148	**2.4**
	96336_at	glycine amidinotransferase (L-arginine:glycine amidinotransferase)	**Gatm**	AI844626	**2.5**
	92217_s_at	glycoprotein 49 B	**Gp49b**	U05265	**2.5**
	92480_f_at	Zinc finger protein 118	**Zfp118**	AB024004	**2.5**
	94458_at	caspase 6	**Casp6**	Y13087	**2.6**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	93497_at	complement component 3	**C3**	K02782	**2.8**
	99178_at	glycoprotein m6b	**Gpm6b**	AI845652	**2.9**
	161281_f_at	immediate early response 3	**Ier3**	AV292869	**3.0**
	97157_at	NK-3 transcription factor, locus 1 (Drosophila)	**Nkx3-1**	U88542	**3.1**
	104580_at	phospholipase C, delta	**Plcd**	U85711	**3.2**
	100325_at	glycoprotein 49 A	**Gp49a**	M65027	**3.2**
	100398_at	Kinesin family member 3a	**Kif3a**	D12645	**3.2**
	104728_at	Protein S (alpha)	**Pros1**	L27439	**3.4**
	100924_at	GATA binding protein 3	**Gata3**	X55123	**3.5**
	92285_at	interleukin 4	**Il4**	AA967539	**3.6**
	103833_at	homeodomain interacting protein kinase 2	**Hipk2**	AF077659	**4.1**
	160374_r_at	polypyrimidine tract binding protein 2	**Ptbp2**	AI119718	**4.2**
	160495_at	aryl-hydrocarbon receptor	**Ahr**	M94623	**5.3**

TABLE 3 (continued)

Target genes of Stat6 in murine CD4+ T cells (Th2 Stat6-/- vs Th2 Stat6+/+)

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
2h	161903_f_at	molecule possessing ankyrin-repeats induced by lipopolysaccharide	**Mail-pending**	AV374591	**-8.0**
	99126_at	inactive X specific transcripts	**Xist**	L04961	**-6.0**
	101920_at	DNA polymerase epsilon, subunit 2	**Pole2**	AF036898	**-5.9**
	101462_r_at	praja1, RING-H2 motif containing	**Pja1**	U06944	**-4.3**
	160948_at	protein phosphatase 3, catalytic subunit, gamma isoform	**Ppp3cc**	M81475	**-4.3**
	103389_at	lysine oxoglutarate reductase, saccharopine dehydrogenase	**Lorsdh**	AJ224761	**-3.4**
	100606_at	prion protein	**Prnp**	M18070	**-3.0**
	162490_f_at	programmed cell death 6	**Pdcd6**	AV138382	**-2.6**
	104663_at	phosphatidylinositol-4-phosphate 5-kinase, type 1 beta	**Pip5k1b**	D86177	**-2.5**
	162313_f_at	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3	**GalNt3**	AV055653	**-2.2**
	160662_r_at	GATA binding protein 6	**Gata6**	AA667100	**-2.2**
	94018_at	ubiquitin-like 3	**Ubl3**	AW120725	**-2.2**
	94321_at	keratin complex 1, acidic, gene 10	**Krt1-10**	V00830	**-2.2**
	98853_at	phospholipase A2, group IB, pancreas, receptor	**Pla2g1br**	D30779	**-2.1**
	92283_s_at	interleukin 4	**Il4**	X03532	**-2.1**
	160399_r_at	H2A histone family, member Y	**H2afy**	AA646966	**-2.1**
	96515_at	interleukin-four induced gene 1	**Fig1**	U70430	**-2.1**
	102024_at	nuclear receptor coactivator 3	**Ncoa3**	AF000581	**-2.1**
	AFFX-MurIL4_at	interleukin 4	**Il4**	M25892	**-2.1**
	96013_r_at	matrin 3	**Matr3**	AI835367	**-2.1**
	95511_at	integrin alpha 6	**Itga6**	X69902	**-2.0**
	102021_at	interleukin 4 receptor, alpha	**Il4ra**	M27960	**-2.0**
	92286_g_at	interleukin 4	**Il4**	AA967539	**-2.0**
	99630_at	mitochondrial ribosomal protein L54	**Mrpl54**	AW060257	**-2.0**
	162379_r_at	vimentin	**Vim**	AV245272	**-1.9**
	100277_at	inhibin beta-A	**Inhba**	X69619	**-1.9**
	99917_at	enhancer of zeste homolog 2 (Drosophila)	**Ezh2**	U52951	**-1.9**
	103021_r_at	mitogen activated protein kinase kinase kinase 1	**Map3k1**	AI317205	**-1.9**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	95784_at	paired-Ig-like receptor A1	**Pira1**	U96682	**2.1**
	104371_at	diacylglycerol O-acyltransferase 1	**Dgat1**	AF078752	**2.3**
	92368_at	receptor (calcitonin) activity modifying protein 3	**Ramp3**	AJ250491	**2.3**
	98525_f_at	erythroid differentiation regulator	**edr**	AJ007909	**2.4**
	94755_at	interleukin 1 alpha	**Il1a**	M14639	**2.5**
	95738_at	pyrroline-5-carboxylate synthetase (glutamate gamma-semialdehyde synthetase)	**Pycs**	AW124889	**2.5**
	92330_r_at	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 1	**Ndufa1**	AA517665	**2.6**
	95546_g_at	insulin-like growth factor 1	**Igf1**	X04480	**3.9**
	160502_at	cellular repressor of E1A-stimulated genes	**Creg**	AF084524	**5.0**
	93638_s_at	immunoglobulin lambda chain, variable 1	**Igl-V1**	J00579	**13.4**
	162172_f_at	neural precursor cell expressed, developmentally down-regulated gene 4a	**Nedd4a**	AV365271	**13.9**
6h	104728_at	protein S (alpha)	**Pros1**	L27439	**-15.3**
	94458_at	caspase 6	**Casp6**	Y13087	**-11.3**
	104228_at	CD84 antigen	**Cd84**	AA607237	**-4.8**
	102021_at	interleukin 4 receptor, alpha	**Il4ra**	M27960	**-2.9**
	94331_at	signal transducer and activator of transcription 6	**Stat6**	L47650	**-2.6**
	98868_at	B-cell leukemia/lymphoma 2	**Bcl2**	L31532	**-2.2**
	92638_at	protein phosphatase 2a, catalytic subunit, alpha isoform	**Ppp2ca**	Z67745	**-2.1**
	96515_at	interleukin-four induced gene 1	**Fig1**	U70430	**-2.1**
	160948_at	protein phosphatase 3, catalytic subunit, gamma isoform	**Ppp3cc**	M81475	**-2.0**
	96533_at	ATP-dependant interferon responsive	**Adir**	AI508931	**1.9**
	96498_at	disrupted meiotic cDNA 1 homolog	**Dmc1h**	D64107	**1.9**
	94781_at	hemoglobin alpha, adult chain 1	**Hba-a1**	V00714	**2.0**
	101144_at	interleukin 18 receptor 1	**Il18r1**	U43673	**2.0**
	99107_at	growth hormone receptor	**Ghr**	M31680	**2.0**
	93078_at	lymphocyte antigen 6 complex, locus A	**Ly6a**	X04653	**2.0**
	162457_f_at	hemoglobin, beta adult major chain	**Hbb-b1**	AV003378	**2.1**
	103534_at	hemoglobin, beta adult minor chain	**Hbb-b2**	V00722	**2.1**
	161894_r_at	zinc finger protein 162	**Zfp162**	AV103574	**2.2**
	92441_at	fibroblast activation protein	**Fap**	Y10007	**2.2**
	94774_at	interferon activated gene 202A	**Ifi202a**	M31418	**2.3**
	97890_at	serum/glucocorticoid regulated kinase	**Sgk**	AW046181	**2.3**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	93858_at	small inducible cytokine B subfamily (Cys-X-Cys), member 10	**Scyb10**	M33266	**2.4**
	103486_at	interleukin 1 beta	**Il1b**	M15131	**2.5**
	97949_at	fibrinogen-like protein 2	**Fgl2**	M16238	**2.9**
	102621_at	cell adhesion molecule-related/down-regulated by oncogenes	**Cdon**	AF090866	**3.5**
	92369_at	transforming growth factor alpha	**Tgfa**	M92420	**5.8**
	161035_at	kinesin 9	**Kif9**	AA122519	**12.4**
24h	99895_at	chemokine (C-C) receptor 8	**Cmkbr8**	AF001277	**-14.5**
	92382_at	myosin VI	**Myo6**	U49739	**-14.4**
	103639_at	interferon-induced protein with tetratricopeptide repeats 2	**Ifit2**	U43085	**-12.9**
	104728_at	protein S (alpha)	**Pros1**	L27439	**-6.2**
	94458_at	caspase 6	**Casp6**	Y13087	**-6.0**
	100924_at	GATA binding protein 3	**Gata3**	X55123	**-5.9**
	93750_at	gelsolin	**Gsn**	J04953	**-5.4**
	93214_at	calcium/calmodulin-dependent protein kinase II, delta	**Camk2d**	AF059029	**-5.2**
	94331_at	signal transducer and activator of transcription 6	**Stat6**	L47650	**-4.9**
	103833_at	homeodomain interacting protein kinase 2	**Hipk2**	AF077659	**-4.2**
	102021_at	interleukin 4 receptor, alpha	**Il4ra**	M27960	**-3.2**
	104155_f_at	activating transcription factor 3	**Atf3**	U19118	**-3.1**
	102955_at	nuclear factor, interleukin 3, regulated	**Nfil3**	U83148	**-3.0**
	100127_at	cellular retinoic acid binding protein II	**Crabp2**	M35523	**-2.9**
	101902_at	recombining binding protein suppressor of hairless (Drosophila)	**Rbpsuh**	X17459	**-2.8**
	160948_at	protein phosphatase 3, catalytic subunit, gamma isoform	**Ppp3cc**	M81475	**-2.6**
	AFFX-MurIL4_at	interleukin 4	**Il4**	M25892	**-2.5**
	92250_s_at	papillary renal cell carcinoma (translocation-associated)	**Prcc**	AA089181	**-2.4**
	92283_s_at	interleukin 4	**Il4**	X03532	**-2.4**
	92286_g_at	interleukin 4	**Il4**	AA967539	**-2.4**
	94821_at	X-box binding protein 1	**Xbp1**	AW123880	**-2.2**
	101945_g_at	lysophospholipase 1	**Lypla1**	U89352	**-2.1**
	104156_r_at	activating transcription factor 3	**Atf3**	U19118	**-2.1**
	103516_at	cadherin EGF LAG seven-pass G-type receptor 1	**Celsr1**	AF031572	**-2.1**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	98282_at	inducible T-cell co-stimulator	**Icos**	AB023132	**-2.1**
	100606_at	prion protein	**Prnp**	M18070	**-2.1**
	103259_at	growth factor independent 1	**Gfi1**	U58972	**-2.1**
	101947_at	neighbor of A-kinase anchoring protein 95	**Nakap95-pending**	AB028921	**-2.1**
	92234_at	retinoid X receptor alpha	**Rxra**	X66223	**-2.1**
	98038_at	high mobility group box 3	**Hmgb3**	AF022465	**-2.0**
	93444_at	basic leucine zipper transcription factor, ATF-like	**Batf**	AF017021	**-2.0**
	92480_f_at	Zinc finger protein 118	**Zfp118**	AB024004	**-2.0**
	99334_at	interferon gamma	**Ifng**	K00083	**1.9**
	102629_at	tumor necrosis factor	**Tnf**	D84196	**2.0**
	98002_at	interferon concensus sequence binding protein	**Icsbp**	M32489	**2.0**
	160906_i_at	ectodermal-neural cortex 1	**Enc1**	AA184423	**2.0**
	94781_at	hemoglobin alpha, adult chain 1	**Hba-a1**	V00714	**2.1**
	94028_f_at	CD84 antigen	**Cd84**	AI642245	**2.1**
	161528_r_at	DNA polymerase delta 1, catalytic domain	**Pold1**	AV227261	**2.1**
	92614_at	inhibitor of DNA binding 3	**Idb3**	M60523	**2.1**
	161167_r_at	uridine monophosphate kinase	**Umpk**	AV223645	**2.1**
	94027_at	CD84 antigen	**Cd84**	AA815831	**2.1**
	101869_s_at	hemoglobin, beta adult major chain	**Hbb-b1**	J00413	**2.2**
	97426_at	epithelial membrane protein 1	**Emp1**	X98471	**2.2**
	103279_at	SH2 domain protein 1A	**Sh2d1a**	AF097632	**2.2**
	162409_r_at	nuclear protein 220	**Np220**	AV315224	**2.3**
	96764_at	interferon-inducible GTPase	**Iigp-pending**	AJ007971	**2.3**
	161884_r_at	fragile X mental retardation gene 1, autosomal homolog	**Fxr1h**	AV368725	**2.3**
	103534_at	hemoglobin, beta adult minor chain	**Hbb-b2**	V00722	**2.3**
	94378_at	regulator of G-protein signaling 16	**Rgs16**	U94828	**2.3**
	94774_at	interferon activated gene 202A	**Ifi202a**	M31418	**2.3**
	98577_f_at	prefoldin 5	**Pfdn5**	Y12713	**2.4**
	97185_at	aryl hydrocarbon receptor nuclear translocator	**Arnt**	AI451564	**2.5**
	102424_at	small inducible cytokine A3	**Scya3**	J04491	**2.5**
	98782_at	complexin 2	**Cplx2**	D38613	**2.7**
	161609_at	regulator of G-protein signaling 16	**Rgs16**	AV349152	**2.7**
	101571_g_at	insulin-like growth factor binding protein 4	**Igfbp4**	X76066	**2.9**
	94146_at	small inducible cytokine A4	**Scya4**	X62502	**3.0**
	93321_at	interferon activated gene 203	**Ifi203**	AF022371	**3.1**
	101313_r_at	Mus musculus endogenous provirus Imposon1		U95783	**3.4**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
		envelope gene, partial cds, and 3' long terminal repeat, complete sequence			
	103389_at	lysine oxoglutarate reductase, saccharopine dehydrogenase	**Lorsdh**	AJ224761	**3.4**
	101144_at	interleukin 18 receptor 1	**Il18r1**	U43673	**4.4**
	161968_f_at	chemokine (C-C) receptor 5	**Cmkbr5**	AV370035	**7.9**
	160726_at	quaking	**qk**	U44940	**11.5**
	102958_at	early B-cell factor 2	**Ebf2**	U82441	**14.8**
	161417_r_at	neuroblastoma myc-related oncogene 1	**Nmyc1**	AV320040	**16.4**
	162451_r_at	f-box only protein 3	**Fbxo3**	AV027999	**33.6**
48h	92382_at	myosin VI	**Myo6**	U49739	**-8.6**
	104728_at	Protein S (alpha)	**Pros1**	L27439	**-4.6**
	103833_at	homeodomain-interacting protein kinase 2(HIPK2)	**Hipk2**	AF077659	**-4.6**
	94331_at	signal transducer and activator of transcription 6	**Stat6**	L47650	**-3.5**
	92286_g_at	Interleukin 4	**Il4**	AA967539	**-3.5**
	100924_at	GATA3	**Gata3**	X55123	**-3.2**
	100325_at	Glycoprotein 49 A (Gp49a)	**Gp49a**	M65027	**-3.2**
	AFFX-MurIL4_at	Interleukin 4	**Il4**	M25892	**-3.2**
	101926_at	protein-serine/threonine kinase (pim-2)	**Pim2**	L41495	**-2.8**
	99178_at	glycoprotein m6b	**Gpm6b**	AI845652	**-2.6**
	100596_at	selenium binding protein 1	**Selenbp1**	M32032	**-2.6**
	93574_at	serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor). member 1	**Serpinf1**	AF036164	**-2.5**
	100606_at	Prion Protein	**Prnp**	M18070	**-2.0**
	102779_at	growth arrest and DNA-damage-inducible 45 beta	**Gadd45b**	X54149	**-2.0**
	160948_at	protein phosphatase 3, catalytic subunit, gamma isoform	**Ppp3cc**	M81475	**-2.0**
	92480_f_at	Zinc finger protein 118	**Zfp118**	AB024004	**-2.0**
	102955_at	NFIL3/E4BP4 transcription factor	**Nfil3**	U83148	**-2.0**
	93865_s_at	histocompatibility 2, T region locus 22	**H2-T22**	M35244	**2.0**
	103025_at	Moloney leukemia virus 10	**Mov10**	X52574	**2.0**
	98600_at	S100 calcium binding protein A11	**S100a11**	U41341	**2.0**
	97507_at	peptidylprolyl isomerase C-associated protein	**Ppicap**	X67809	**2.1**
	104679_at	TXK tyrosine kinase	**Txk**	D43963	**2.1**
	98525_f_at	erythroid differentiation regulator	**edr**	AJ007909	**2.1**
	98030_at	tripartite motif protein 30	**Trim30**	J03776	**2.3**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	98822_at	interferon-stimulated protein (15 kDa)	**Isg15**	X56602	**2.5**
	92644_s_at	myb proto-oncogene	**Myb**	M12848	**2.5**
	93078_at	lymphocyte antigen 6 complex	**Ly6a**	X04653	**2.5**
	102877_at	granzyme B (Gzmb)	**Gzmb**	M12302	**2.6**
	92214_at	cathepsin W	**Ctsw**	AF014941	**2.6**
	102906_at	T-cell specific GTPase	**Tgtp**	L38444	**2.8**
	96060_at	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6	**Serpinb6**	U25844	**2.8**
	93321_at	interferon activated gene 203	**Ifi203**	AF022371	**3.5**
	103554_at	a disintegrin and metalloproteinase domain 19 (meltrin beta)	**Adam19**	AA726223	**4.0**
	161968_f_at	chemokine (C-C) receptor 5	**Cmkbr5**	AV370035	**4.3**
	94224_s_at	interferon activated gene 205	**Ifi205**	M74123	**4.6**
	101144_at	interleukin 18 receptor 1	**Il18r1**	U43673	**4.9**
	103432_at	interferon-stimulated protein (20 kDa)	**Isg20**	AW122677	**5.7**

Table IV. The human oligos used for quantitative Real-Time RT-PCR.

ACCESSION	GENE	1) 5'- 6(FAM)-PROBE-(TAMRA)-3' 2) 5' -PRIMER 1- 3' 3) 5' -PRIMER 2 -3'
AB013602, AB013382	DUSP6 (short & long)	1)5'-CTCTACGACGAGAGCAGCAGCGACTG-3' 2) 5'-GCTGTGGCACCGACACAGT-3' 3) 5'-ACTCGCCGCCCGTATTCT-3'
AB013382	DUSP6 (long)	1)5'-CTCTACGACGAGAGCAGCAGCGACTG-3' 2) 5'-GCTGTGGCACCGACACAGT-3' 3) 5'-GAACTCGGCTTGGAACTTACTGAA-3'
X64318	E4BP4 (NFIL3)	1) 5'-TCCTCAGTAGAACACACGCAGGAGAGCTC-3' 2) 5´-AGCTCGCTGTCCGATGTTTC-3' 3) 5'-CTTCTGCAGCTTCCCTGCAC-3
J04617	EF1α	1) 5' -AGCGCCGGCTATGCCCCTG- 3' 2) 5' -CTGAACCATCCAGGCCAAAT- 3' 3) 5' -GCCGTGTGGCAATCCAAT- 3'
AF078077	GADD45β (Myd118)	1) 3'-TGGCCACCTCCACCAAGCCG-3' 2) 3'-CCGGCTTTCTTCGCAGTAG-5' 3) 3'-CACGGACGCCTGGAAGA-5'
X55122	GATA-3	1) 5'-TGCCGGAGGAGGTGGATGTGCT-3' 2) 5'-GGACGCGGCGCAGTAC-3' 3) 5'-TGCCTTGACCGTCGATGTTA-3'
M37265	IFNγ	1) 5'-TGCTGGCGACAGTTCAGCCATCAC-3' 2) 5'-CTCGAAACAGCATCTGACTCCTT-3' 3) 5'-TGTCCAACGCAAAGCAATACA-3'
m97287	SATB1	1) 5´-AACGAGCAGGAATCTCCCAGGCG-3' 2) 5'-ACCAGTGGGTACGCGATGA-3' 3) 5'-TGTTAAAAGCCACACGTGCAA- 3'
AF241243	T-bet	1) 5' -TCAGCATGAAGCCTGCATTCTTGCC- 3' 2) 5' -ACAGCTATGAGGCTGAGTTTCGA- 3' 3) 5' -GGCCTCGGTAGTAGGACATGG T- 3'
AB000734	TIP3 (SSI-1, SOCS-1, JAB)	1) 5'-TTCGCACGCCGATTACCGGC-'3 2) 5'-ACACGCACTTCCGCACATT-3' 3) 5'-CTGGCGCGCGTGATG-'3

Table V: The murine oligos used for quantitative Real-Time RT-PCR.

	Forward Primer	Reverse Primer	Probe
Hipk2	CAGTGAAGTGTTGGTAGAATGTGACA	GCTGGAGGACTTGGACTTGAAG	CCAGCGATCAGTGCCAGTCACCATT
Nfil3	CATCACAAAGAACTGAGCAGCAA	AACCTTATAGCCACCGTCTTTGAC	TCCACCACACCTGTTTTGAAGCTACTCTGAG
Zfp118	GAACAAAGAGACCTGGAAGATGGA	GAAGAATAATGAATAGCTGGCTTGTG	TCCTGGATCCTTAGCTACTGCCTCCTGTCT
Atf3	GGCGGCGAGAAAGAAATAAA	GCAGGCACTCTGTCTTCTCCTT	TCTTGTTTCGACACTTGGCAGCAGCA
Ifi203	TCAGCTGGCGGACTGGAT	TGCTCACACACTTTTATCAGTTTGTC	AGGACAAGTTCCCCAAAGATGCTGGACT

TABLE 6

Novel target genes of IL-4 in murine CD4+ T cells (Th2-induced vs activated)

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
2h	103841_at	zinc finger protein 64	**Zfp64**	U49046	**-3.5**
	160273_at	zinc finger protein 36, C3H type-like 2	**Zfp36l2**	AA960603	**-2.3**
	96623_at	UDP-glucose ceramide glucosyltransferase-like	**Ugcgl**	AI853172	**-2.3**
	92368_at	receptor (calcitonin) activity modifying protein 3	**Ramp3**	AJ250491	**-2.2**
	98478_at	cyclin G2	**Ccng2**	U95826	**-2.1**
	98072_r_at	deoxycytidine kinase	**Dck**	X77731	**-2.0**
	104371_at	diacylglycerol O-acyltransferase 1	**Dgat1**	AF078752	**-1.9**
	160439_at	polymerase, gamma	**Polg**	U53584	**2.0**
	102797_at	retinal short-chain dehydrogenase/reductase 1	**Rsdr1-pending**	X95281	**2.0**
	104449_at	glycine receptor, beta subunit	**Glrb**	X81202	**2.2**
	104400_at	prenylated SNARE protein	**Ykt6-pending**	AF076956	**2.3**
	100606_at	prion protein	**Prnp**	M18070	**2.3**
	99021_at	paired related homeobox 1	**Prrx1**	U03873	**2.5**
	100022_at	cytokine inducible SH2-containing protein	**Cish**	D89613	**2.5**
	99917_at	enhancer of zeste homolog 2 (Drosophila)	**Ezh2**	U52951	**2.5**
	97523_i_at	amylase 2, pancreatic	**Amy2**	X02578	**2.6**
	160948_at	protein phosphatase 3, catalytic subunit, gamma isoform	**Ppp3cc**	M81475	**2.8**
	161411_i_at	lipase, hormone sensitive	**Lipe**	AV315398	**8.1**
	161491_r_at	f-box only protein 3	**Fbxo3**	AV083174	**10.5**
	101920_at	DNA polymerase epsilon, subunit 2	**Pole2**	AF036898	**15.1**
6h	100131_at	secretory granule neuroendocrine protein 1, 7B2 protein	**Sgne1**	X15830	**-6.0**
	102157_f_at	immunoglobulin kappa chain variable 28 (V28)	**Igk-V28**	M15520	**-4.7**
	161997_f_at	aldehyde dehydrogenase 2, mitochondrial	**Aldh2**	AV329607	**-2.9**
	92280_at	actinin, alpha 1	**Actn1**	AA867778	**-2.2**
	92608_at	cysteine rich protein	**Csrp**	D88793	**-2.1**
	101965_at	ring finger protein 13	**Rnf13**	AF037205	**-2.0**
	92614_at	inhibitor of DNA binding 3	**Idb3**	M60523	**-2.0**
	100022_at	cytokine inducible SH2-containing protein	**Cish**	D89613	**2.0**
	101913_at	hairy/enhancer-of-split related with YRPW motif 1	**Hey1**	AW214298	**2.6**
	161051_at	hairy and enhancer of split 5, (Drosophila)	**Hes5**	D32132	**2.6**
	160886_i_at	fatty acid binding protein 6, ileal (gastrotropin)	**Fabp6**	AV063979	**3.9**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	100127_at	cellular retinoic acid binding protein II	**Crabp2**	M35523	**5.1**
24h	160726_at	Quaking	**qk**	U44940	**-11.4**
	161609_at	regulator of G-protein signaling 16	**Rgs16**	AV349152	**-2.5**
	96060_at	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6	**Serpinb6**	U25844	**-2.3**
	161948_f_at	myosin Va	**Myo5a**	AV214912	**-2.0**
	102638_at	Cystatin F (leukocystatin)	**Cst7**	AB015224	**2.0**
	92234_at	Retinoid X receptor alpha	**Rxra**	X66223	**2.0**
	161012_at	immunoglobulin-associated beta	**Igb**	J03857	**2.1**
	93444_at	basic leucine zipper transcription factor, ATF-like	**Batf**	AF017021	**2.3**
	101902_at	recombining binding protein suppressor of hairless (Drosophila)	**Rbpsuh**	X17459	**2.5**
	93750_at	Gelsolin	**Gsn**	J04953	**3.9**
	92382_at	myosin VI	**Myo6**	U49739	**5.3**
48h	93880_at	eomesodermin homolog (Xenopus laevis)	**Eomes**	AB031037	**-5.1**
	103554_at	a disintegrin and metalloproteinase domain 19 (meltrin beta)	**Adam19**	AA726223	**-4.3**
	160878_at	block of proliferation 1	**Bop1**	AF061503	**-4.2**
	96060_at	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6	**Serpinb6**	U25844	**-4.2**
	99461_at	hematopoietic cell specific Lyn substrate 1	**Hcls1**	X84797	**-3.5**
	98446_s_at	Eph receptor B4	**Ephb4**	U06834	**-3.4**
	102635_at	vesicle transport through interaction with t-SNAREs 1 homolog	**Vti1-pending**	AF035823	**-3.3**
	94855_at	prohibitin	**Phb**	X78682	**-3.2**
	104093_at	lymphocyte specific 1	**Lsp1**	D49691	**-3.1**
	103367_at	UDP-N-acetyl-alpha-D-galactosamine:(N-acetylneuraminyl)-galactosylglucosylceramide-beta-1, 4-N-acetylgalactosaminyltransferase	**Galgt1**	U18975	**-2.9**
	96046_at	histone deacetylase 1	**Hdac1**	X98207	**-2.9**
	97507_at	peptidylprolyl isomerase C-associated protein	**Ppicap**	X67809	**-2.9**
	96297_at	EBNA1 binding protein 2	**Ebp2**	AI845934	**-2.8**
	93218_at	SWAP complex protein, 70 kDa	**Swap70**	AF053974	**-2.8**
	92220_s_at	myc box dependent interacting protein 1	**Bin1**	U60884	**-2.8**
	98589_at	Adipose differentiation related protein	**Adfp**	M93275	**-2.6**
	97875_at	adhesion regulating molecule 1	**Adrm1**	AW123694	**-2.6**
	93518_at	ribonucleic acid binding protein S1	**Rnps1**	X70067	**-2.5**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	160944_at	plakophilin 3	**Pkp3**	AW120906	**-2.4**
	95733_at	solute carrier family 29 (nucleoside transporters), member 1	**Slc29a1**	AI838274	**-2.4**
	92879_at	protein phosphatase 1G (formerly 2C), magnesium-dependent, gamma isoform	**Ppm1g**	U42383	**-2.4**
	161585_at	glycoprotein 5 (platelet)	**Gp5**	AV318587	**-2.4**
	160318_at	STIP1 homology and U-Box containing protein 1	**Stub1**	AI844511	**-2.4**
	103823_at	topoisomerase (DNA) III beta	**Top3b**	AB013603	**-2.4**
	99486_at	centromere autoantigen B	**Cenpb**	X55038	**-2.4**
	94815_at	2,3-bisphosphoglycerate mutase	**Bpgm**	X13586	**-2.4**
	97251_at	mitochondrial ribosomal protein S10	**Mrps10**	AI842124	**-2.4**
	104275_g_at	transformation related protein 53	**Trp53**	AB021961	**-2.4**
	100632_at	protein kinase, AMP-activated, gamma 1 non-catalytic subunit	**Prkag1**	AF036535	**-2.3**
	160106_at	Capping protein (actin filament), gelsolin-like	**Capg**	X54511	**-2.3**
	104025_at	thimet oligopeptidase 1	**Thop1**	AW047185	**-2.3**
	99639_at	ubiquintin c-terminal hydrolase related polypeptide	**Uchrp**	D84096	**-2.3**
	92637_at	phosphofructokinase, liver, B-type	**Pfkl**	J03928	**-2.3**
	93364_at	catenin alpha 1	**Catna1**	X59990	**-2.2**
	93892_at	CUG triplet repeat,RNA binding protein 2	**Cugbp2**	Y18298	**-2.2**
	103025_at	Moloney leukemia virus 10	**Mov10**	X52574	**-2.2**
	99669_at	lectin, galactose binding, soluble 1	**Lgals1**	X15986	**-2.2**
	104653_at	core binding factor beta	**Cbfb**	AA864065	**-2.2**
	97532_at	protein phosphatase 5, catalytic subunit	**Ppp5c**	AF018262	**-2.1**
	101989_at	ubiquinol-cytochrome c reductase core protein 1	**Uqcrc1**	AW125380	**-2.1**
	98030_at	Tripartite motif protein 30	**Trim30**	J03776	**-2.1**
	98339_at	synaptotagmin 11	**Syt11**	AB026808	**-2.1**
	100101_at	small nuclear ribonucleoprotein polypeptide A	**Snrpa**	L15447	**-2.1**
	92540_f_at	spermidine synthase	**Srm**	Z67748	**-2.1**
	98153_at	chaperonin subunit 3 (gamma)	**Cct3**	L20509	**-2.1**
	160290_at	insulin degrading enzyme	**Ide**	AI574278	**-2.1**
	97867_at	hydroxysteroid 11-beta dehydrogenase 1	**Hsd11b1**	X83202	**-2.1**
	99169_at	coactivator-associated arginine methyltransferase 1	**Carm1-pending**	AW122165	**-2.0**
	96883_at	eukaryotic translation initiation factor 3, subunit 4 (delta, 44 kDa)	**Eif3s4**	U70733	**-2.0**
	101492_at	protein (peptidyl-prolyl cis/trans isomerase) NIMA-	**Pin1**	AW047032	**-2.0**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
		interacting 1			
	102257_at	Pbx/knotted 1 homeobox	**Pknox1**	AF061270	**-2.0**
	93500_at	aminolevulinic acid synthase 1	**Alas1**	M63245	**-2.0**
	103683_at	dihydroorotate dehydrogenase	**Dhodh**	AF029667	**-2.0**
	99478_at	protein tyrosine phosphatase, receptor type, C polypeptide-associated protein	**Ptprcap**	X97268	**-2.0**
	103946_at	proline-serine-threonine phosphatase-interacting protein 1	**Pstpip1**	U87814	**-2.0**
	104137_at	ATP-binding cassette, sub-family A (ABC1), member 2	**Abca2**	X75927	**-2.0**
	100156_at	mini chromosome maintenance deficient 5 (S. cerevisiae)	**Mcmd5**	D26090	**-2.0**
	104102_at	protease, serine, 25	**Prss25**	AW047978	**-2.0**
	94138_s_at	fucosyltransferase 7	**Fut7**	U45980	**-2.0**
	94850_at	acyl-Coenzyme A thioesterase 3, mitochondrial	**Acate3-pending**	AJ238894	**-1.9**
	103518_at	cytotoxic T lymphocyte-associated protein 2 beta	**Ctla2b**	X15592	**2.0**
	160439_at	polymerase, gamma	**Polg**	U53584	**2.0**
	100606_at	prion protein	**Prnp**	M18070	**2.0**
	99143_at	trans-golgi network protein 2	**Tgoln2**	AA614914	**2.2**
	96912_s_at	cytotoxic T lymphocyte-associated protein 2 alpha	**Ctla2a**	X15591	**2.2**
	162172_f_at	neural precursor cell expressed, developmentally down-regulated gene 4a	**Nedd4a**	AV365271	**2.2**
	96336_at	glycine amidinotransferase (L-arginine:glycine amidinotransferase)	**Gatm**	AI844626	**2.5**
	92217_s_at	glycoprotein 49 B	**Gp49b**	U05265	**2.5**
	92480_f_at	Zinc finger protein 118	**Zfp118**	AB024004	**2.5**
	99178_at	glycoprotein m6b	**Gpm6b**	AI845652	**2.9**
	161281_f_at	immediate early response 3	**Ier3**	AV292869	**3.0**
	97157_at	NK-3 transcription factor, locus 1 (Drosophila)	**Nkx3-1**	U88542	**3.1**
	100325_at	glycoprotein 49 A	**Gp49a**	M65027	**3.2**
	160374_r_at	polypyrimidine tract binding protein 2	**Ptbp2**	AI119718	**4.2**
	160495_at	aryl-hydrocarbon receptor	**Ahr**	M94623	**5.3**

TABLE 6 (continued)

Novel target genes of Stat6 in murine CD4+ T cells (Th2-induced Stat6-/- cells vs Th2-induced Stat6+/+ cells)

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
2h	161903_f_at	molecule possessing ankyrin-repeats induced by lipopolysaccharide	**Mail-pending**	AV374591	**-8.0**
	99126_at	inactive X specific transcripts	**Xist**	L04961	**-6.0**
	101920_at	DNA polymerase epsilon, subunit 2	**Pole2**	AF036898	**-5.9**
	101462_r_at	praja1, RING-H2 motif containing	**Pja1**	U06944	**-4.3**
	160948_at	protein phosphatase 3, catalytic subunit, gamma isoform	**Ppp3cc**	M81475	**-4.3**
	103389_at	lysine oxoglutarate reductase, saccharopine dehydrogenase	**Lorsdh**	AJ224761	**-3.4**
	100606_at	prion protein	**Prnp**	M18070	**-3.0**
	162490_f_at	programmed cell death 6	**Pdcd6**	AV138382	**-2.6**
	104663_at	phosphatidylinositol-4-phosphate 5-kinase, type 1 beta	**Pip5k1b**	D86177	**-2.5**
	162313_f_at	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3	**Galnt3**	AV055653	**-2.2**
	160662_r_at	GATA binding protein 6	**Gata6**	AA667100	**-2.2**
	94018_at	ubiquitin-like 3	**Ubl3**	AW120725	**-2.2**
	94321_at	keratin complex 1, acidic, gene 10	**Krt1-10**	V00830	**-2.2**
	98853_at	phospholipase A2, group IB, pancreas, receptor	**Pla2g1br**	D30779	**-2.1**
	160399_r_at	H2A histone family, member Y	**H2afy**	AA646966	**-2.1**
	102024_at	nuclear receptor coactivator 3	**Ncoa3**	AF000581	**-2.1**
	96013_r_at	matrin 3	**Matr3**	AI835367	**-2.1**
	95511_at	integrin alpha 6	**Itga6**	X69902	**-2.0**
	99630_at	mitochondrial ribosomal protein L54	**Mrpl54**	AW060257	**-2.0**
	162379_r_at	vimentin	**Vim**	AV245272	**-1.9**
	99917_at	enhancer of zeste homolog 2 (Drosophila)	**Ezh2**	U52951	**-1.9**
	100277_at	inhibin beta-A	**Inhba**	X69619	**-1.9**
	103021_r_at	mitogen activated protein kinase kinase kinase 1	**Map3k1**	AI317205	**-1.9**
	95784_at	paired-Ig-like receptor A1	**Pira1**	U96682	**2.1**
	104371_at	diacylglycerol O-acyltransferase 1	**Dgat1**	AF078752	**2.3**
	92368_at	receptor (calcitonin) activity modifying protein 3	**Ramp3**	AJ250491	**2.3**
	94755_at	interleukin 1 alpha	**Il1a**	M14639	**2.5**
	95738_at	pyrroline-5-carboxylate synthetase (glutamate	**Pycs**	AW124889	**2.5**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
		gamma-semialdehyde synthetase)			
	92330_r_at	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 1	**Ndufa1**	AA517665	**2.6**
	160502_at	cellular repressor of E1A-stimulated genes	**Creg**	AF084524	**5.0**
	93638_s_at	immunoglobulin lambda chain, variable 1	**Igl-V1**	J00579	**13.4**
	162172_f_at	neural precursor cell expressed, developmentally down-regulated gene 4a	**Nedd4a**	AV365271	**13.9**
6h	104228_at	CD84 antigen	**Cd84**	AA607237	**-4.8**
	92638_at	protein phosphatase 2a, catalytic subunit, alpha isoform	**Ppp2ca**	Z67745	**-2.1**
	160948_at	protein phosphatase 3, catalytic subunit, gamma isoform	**Ppp3cc**	M81475	**-2.0**
	96533_at	ATP-dependant interferon responsive	**Adir**	AI508931	**1.9**
	96498_at	disrupted meiotic cDNA 1 homolog	**Dmc1h**	D64107	**1.9**
	94781_at	hemoglobin alpha, adult chain 1	**Hba-a1**	V00714	**2.0**
	99107_at	growth hormone receptor	**Ghr**	M31680	**2.0**
	162457_f_at	hemoglobin, beta adult major chain	**Hbb-b1**	AV003378	**2.1**
	103534_at	hemoglobin, beta adult minor chain	**Hbb-b2**	V00722	**2.1**
	161894_r_at	zinc finger protein 162	**Zfp162**	AV103574	**2.2**
	92441_at	fibroblast activation protein	**Fap**	Y10007	**2.2**
	97890_at	serum/glucocorticoid regulated kinase	**Sgk**	AW046181	**2.3**
	102621_at	cell adhesion molecule-related/down-regulated by oncogenes	**Cdon**	AF090866	**3.5**
	92369_at	transforming growth factor alpha	**Tgfa**	M92420	**5.8**
	161035_at	kinesin 9	**Kif9**	AA122519	**12.4**
24h	92382_at	myosin VI	**Myo6**	U49739	**-14.9**
	93750_at	gelsolin	**Gsn**	J04953	**-5.4**
	93214_at	calcium/calmodulin-dependent protein kinase II, delta	**Camk2d**	AF059029	**-5.2**
	100127_at	cellular retinoic acid binding protein II	**Crabp2**	M35523	**-2.9**
	101902_at	recombining binding protein suppressor of hairless (Drosophila)	**Rbpsuh**	X17459	**-2.8**
	160948_at	protein phosphatase 3, catalytic subunit, gamma isoform	**Ppp3cc**	M81475	**-2.6**
	92250_s_at	papillary renal cell carcinoma (translocation-associated)	**Prcc**	AA089181	**-2.4**
	94821_at	X-box binding protein 1	**Xbp1**	AW123880	**-2.2**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	101945_g_at	lysophospholipase 1	**Lypla1**	U89352	**-2.1**
	103516_at	cadherin EGF LAG seven-pass G-type receptor 1	**Celsr1**	AF031572	**-2.1**
	101947_at	neighbor of A-kinase anchoring protein 95	**Nakap95-pending**	AB028921	**-2.1**
	100606_at	prion protein	**Prnp**	M18070	**-2.1**
	92234_at	retinoid X receptor alpha	**Rxra**	X66223	**-2.1**
	98038_at	high mobility group box 3	**Hmgb3**	AF022465	**-2.0**
	93444_at	basic leucine zipper transcription factor, ATF-like	**Batf**	AF017021	**-2.0**
	92480_f_at	Zinc finger protein 118	**Zfp118**	AB024004	**-2.0**
	160906_i_at	ectodermal-neural cortex 1	**Enc1**	AA184423	**2.0**
	94781_at	hemoglobin alpha, adult chain 1	**Hba-a1**	V00714	**2.1**
	94028_f_at	CD84 antigen	**Cd84**	AI642245	**2.1**
	161528_r_at	DNA polymerase delta 1, catalytic domain	**Pold1**	AV227261	**2.1**
	161167_r_at	uridine monophosphate kinase	**Umpk**	AV223645	**2.1**
	94027_at	CD84 antigen	**Cd84**	AA815831	**2.1**
	92614_at	inhibitor of DNA binding 3	**Idb3**	M60523	**2.1**
	97426_at	epithelial membrane protein 1	**Emp1**	X98471	**2.2**
	101869_s_at	hemoglobin, beta adult major chain	**Hbb-b1**	J00413	**2.2**
	162409_r_at	nuclear protein 220	**Np220**	AV315224	**2.3**
	161884_r_at	fragile X mental retardation gene 1, autosomal homolog	**Fxr1h**	AV368725	**2.3**
	103534_at	hemoglobin, beta adult minor chain	**Hbb-b2**	V00722	**2.3**
	94378_at	regulator of G-protein signaling 16	**Rgs16**	U94828	**2.3**
	98577_f_at	prefoldin 5	**Pfdn5**	Y12713	**2.4**
	97185_at	aryl hydrocarbon receptor nuclear translocator	**Arnt**	AI451564	**2.5**
	98782_at	complexin 2	**Cplx2**	D38613	**2.7**
	161609_at	regulator of G-protein signaling 16	**Rgs16**	AV349152	**2.7**
	103389_at	lysine oxoglutarate reductase, saccharopine dehydrogenase	**Lorsdh**	AJ224761	**3.4**
	101313_r_at	Mus musculus endogenous provirus Imposon1 envelope gene, partial cds, and 3' long terminal repeat, complete sequence		U95783	**3.4**
	160726_at	quaking	**qk**	U44940	**11.5**
	102958_at	early B-cell factor 2	**Ebf2**	U82441	**14.8**
	162451_r_at	f-box only protein 3	**Fbxo3**	AV027999	**33.6**
48h	92382_at	myosin VI	**Myo6**	U49739	**-8.6**
	100325_at	Glycoprotein 49 A (Gp49a)	**Gp49a**	M65027	**-3.2**
	101926_at	protein-serine/threonine kinase (pim-2)	**Pim2**	L41495	**-2.8**

TIME	AFFY PROBE ID	GENE NAME	SYMBOL	ACCESSION	FOLD CHANGE
	100596_at	selenium binding protein 1	**Selenbp1**	M32032	**-2.6**
	99178_at	glycoprotein m6b	**Gpm6b**	AI845652	**-2.6**
	93574_at	serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor). member 1	**Serpinf1**	AF036164	**-2.5**
	160948_at	protein phosphatase 3, catalytic subunit, gamma isoform	**Ppp3cc**	M81475	**-2.0**
	100606_at	Prion Protein	**Prnp**	M18070	**-2.0**
	92480_f_at	Zinc finger protein 118	**Zfp118**	AB024004	**-2.0**
	102779_at	growth arrest and DNA-damage-inducible 45 beta	**Gadd45b**	X54149	**-2.0**
	103025_at	Moloney leukemia virus 10	**Mov10**	X52574	**2.0**
	98600_at	S100 calcium binding protein A11	**S100a11**	U41341	**2.0**
	97507_at	peptidylprolyl isomerase C-associated protein	**Ppicap**	X67809	**2.1**
	98030_at	tripartite motif protein 30	**Trim30**	J03776	**2.3**
	92644_s_at	myb proto-oncogene	**Myb**	M12848	**2.5**
	92214_at	cathepsin W	**Ctsw**	AF014941	**2.6**
	96060_at	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6	**Serpinb6**	U25844	**2.8**
	103554_at	a disintegrin and metalloproteinase domain 19 (meltrin beta)	**Adam19**	AA726223	**4.0**

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CLAIMS:

1. A method of identifying a compound capable of modulating the polarization of CD4+ lymphocytes, the method comprising:

(a) contacting said compound with naïve CD4+ lymphocytes;

(b) inducing the polarization of the lymphocytes;

(c) preparing a gene expression profile from the lymphocytes;

(d) comparing the lymphocyte gene expression profile to a gene expression profile derived from Table 1.

2. The method according to claim 1, wherein the induction of step (b) is performed by contacting the lymphocytes with a cytokine.

3. The method according to claim 2, wherein said cytokine is IL-12 or IL-4.

4. The method according to any one of the preceding claims, wherein a difference in the expression profiles of the target genes identifies a potential drug compound for the treatment of asthma or other immune-mediated diseases.

5. A method of identifying a compound that modulates the expression of at least one gene listed in Table 2 or Table 6, the method comprising:

(a) incubating a cell that can express a protein from said gene with a compound under conditions and for a time sufficient for the cell to express the protein of said gene, when the compound is not present;

(b) incubating a control cell under the same conditions and for the same time without the compound;

(c) measuring expression of said gene in the cell in the presence of the compound;

(d) measuring expression of said gene in the control cell; and

(e) comparing the amount of expression of said gene in the presence and absence of the compound, wherein a difference in the level of expression indicates that the compound modulates the expression of said gene.

6. A method of identifying a compound that modulates the activity of at least one gene listed in Table 2 or Table 6, the method comprising:

(a) incubating a cell that has said activity with a compound under conditions and for a time sufficient for the cell to express said activity, when the compound is not present;

(b) incubating a control cell under the same conditions and for the same time without the compound;

(c) measuring said activity in the cell in the presence of the compound;

(d) measuring said activity in the control cell; and

(e) comparing the amount of said activity in the presence and absence of the compound, wherein a difference in the level of expression indicates that the compound modulates the activity of said gene.

7. A method of treating a patient with asthma or other immune-mediated disease, the method comprising administering to the patient a pharmaceutical composition, wherein the composition alters the expression or activity of at least one gene listed in Table 2 or Table 6.

8. The method according to claim 7, wherein the active compound of said pharmaceutical composition is identified by a method of any one of claims 1 - 6.

9. The method according to claim 7, wherein the active compound of said pharmaceutical composition is an antibody binding to at least one gene product of the genes listed in Table 2 or Table 6.

10. A pharmaceutical composition altering the expression or activity of at least one gene listed in Table 2 or Table 6 for use in prophylaxis or treatment of asthma or other immune-mediated disease.

11. The pharmaceutical composition according to claim 9, comprising an antibody binding to at least one gene product of the genes listed in Table 2 or Table 6 as an active ingredient.

12. A method of identifying a compound capable of modulating the polarization of murine CD4+ lymphocytes, the method comprising:

(a) contacting said compound with naïve murine CD4+ lymphocytes;

(b) inducing the polarization of the lymphocytes;

(c) preparing a gene expression profile from the lymphocytes;

comparing the lymphocyte gene expression profile to a gene expression profile derived from Table 3.

13. A method of determining the activity of the compound identified in a method according to any one of claims 1-6 in a mouse model, the method comprising

(a) contacting said compound with naïve murine CD4+ lymphocytes;

(b) inducing the polarization of the lymphocytes;

(c) preparing a gene expression profile from the lymphocytes;

comparing the lymphocyte gene expression profile to a gene expression profile derived from Table 3.

14. A method according to any one of claims 1-4, wherein said gene expression profile derived from Table 1 is at least partly based on the expression fold changes of any one of the genes selected from the group consisting of: KIAA0053, LRRN3, CIG5, DUSP6, FER1L3, S100P, SATB1, SLC11A2, STK17B, a gene identified by accession number AI971169 and a gene identified by accession number AL432401.

15. A method according to any one of claims 5-11, wherein the gene or genes is/are selected from the group consisting of: KIAA0053, LRRN3, CIG5, DUSP6, FER1L3, S100P, SATB1, SLC11A2, STK17B, a gene identified by accession number AI971169 and a gene identified by accession number AL432401.

16. A method according to any of claims 1-4, wherein said lymphocyte gene expression profile and said gene expression profile derived from Table 1 each comprise at least 5 of the same genes listed in Table 1.

17. The method of claim 16, wherein the lymphocyte gene expression profile and the gene expression profile derived from Table 1 each comprise at least 10 of the same genes listed in Table 1.

18. The method of claim 17, wherein the lymphocyte gene expression profile and the gene expression profile derived from Table 1 each comprise at least 25 of the same genes listed in Table 1.

19. The method according to claim 5, wherein measuring steps (c) and (d) each comprise measuring the expression of at least 5 genes from those listed in Table 2 or Table 6

and comparing comprises comparing the expression of these genes in both the cell and control cell.

20. The method according to claim 19, wherein the expression of at least 10 genes is measured in steps (c) and (d).

21. The method according to claim 20, wherein the expression of at least 25 genes is measured in steps (c) and (d).

22. A method according to any of claims 6-11, wherein measuring steps (c) and (d) each comprise measuring the activity of at least 5 genes from those listed in Table 2 or Table 6 and comparing comprises comparing the activity of these genes in both the cell and control cell.

23. The method according to claim 22, wherein the activity of at least 10 genes is measured in steps (c) and (d).

24. The method according to claim 23, wherein the activity of at least 25 genes is measured in steps (c) and (d).

25. A method for classifying a lymphocyte, comprising:

(a) providing a test sample derived from the lymphocyte, wherein the lymphocyte is capable of expressing one or more nucleic acid markers from the group consisting of those listed in Table 2 or Table 6;

(b) determining the expression level of the one or more markers in the test sample;

(c) comparing the expression level of the one or more markers in the test sample with the expression level of the same markers in a control sample, wherein the control sample is derived from a lymphocytic cell whose cellular status is known; and

(d) classifying the lymphocyte on the basis of the comparison of step (c).

26. The method of claim 25, wherein the control sample is derived from a Th1 or Th2 cell and classifying comprises identifying the lymphocyte as a Th1 or Th2 cell.

27. A method for diagnosing the presence of, or a predisposition to, an immune-related disorder in a subject, the method comprising:

(a) determining the expression level of one or more nucleic acid markers in a test sample obtained from the subject, wherein the one or more nucleic acid markers are selected from the group consisting of those listed in Table 2 or Table 6;

(b) comparing the expression level of the one or more nucleic acid markers in the test sample with the expression level of the same markers in a control sample whose immune status is known; and

(c) diagnosing the presence or absence of the immune disorder in the subject, or a predisposition to the immune disorder, on the basis of the comparison of step (b).

28. A method for identifying a compound that modulates differentiation of a lymphocyte, the method comprising:

(a) contacting a test cell capable of expressing one or more gene markers listed in Table 2 or Table 6 with a test compound; and

(b) determining the expression level of the one or more gene markers in the test cell; and

(c) comparing the expression level of the one or more gene markers with the expression level of the same makers for a control cell, wherein

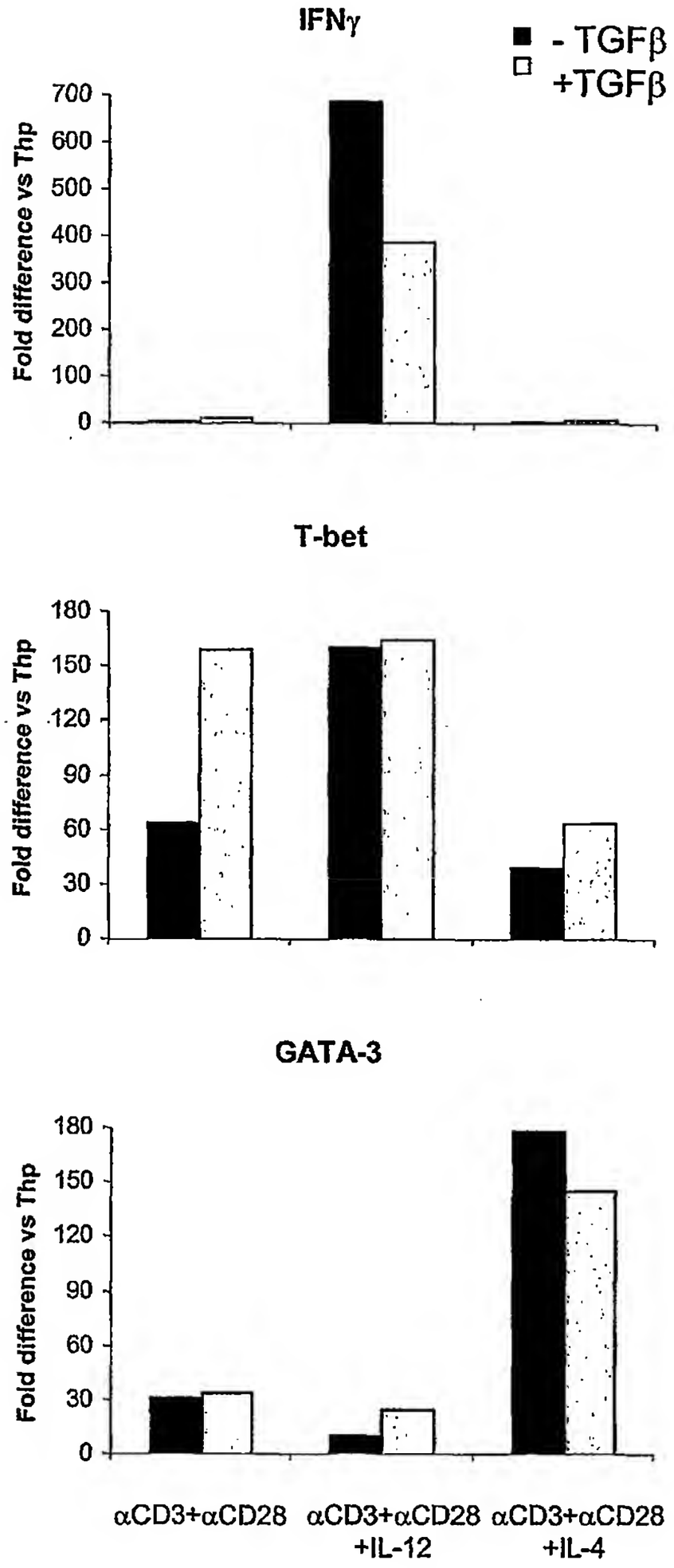
the test cell and the control cell are lymphocytes and the cellular state of the control cell is known; and

a difference in the expression level between the test and control cell is an indicator that the test compound is a modulator of lymphocyte differentiation.

29. The method according to claim 28, wherein the expression level of at least 5 gene markers are determined and compared.

30. The method according to claim 29, wherein the expression level of at least 10 gene markers are determined and compared.

31. The method according to claim 30, wherein the expression level of at least 25 gene markers are determined and compared.


IFNγ
- TGFβ
+TGFβ
Fold difference vs Thp
700
600
500
400
300
200
100
0
T-bet
Fold difference vs Thp
180
150
120
90
60
30
0
GATA-3
Fold difference vs Thp
180
150
120
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60
30
0
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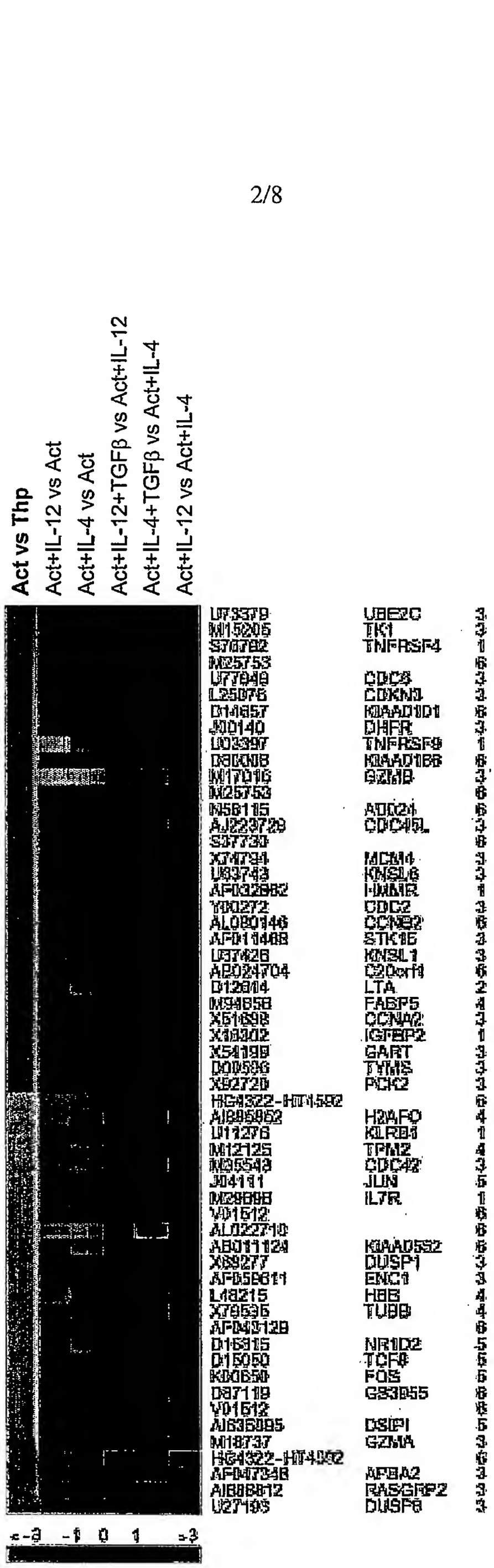
αCD3+αCD28 +IL-12
αCD3+αCD28 +IL-4

Figure 1.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Act+IL-4 vs Act
Act+IL-12+TGFβ vs Act+IL-12
Act+IL-4+TGFβ vs Act+IL-4
Act+IL-12 vs Act+IL-4
U73379 UBE2C
M15205 TK1
S70782 TNFRSF4
M25753
U77949 CDC6
L25076 CDKN3
D14657 KIAA0101
J00140 DHFR
U03397 TNFRSF9
D80008 KIAA0186
M17016 GZMB
M25753
N56115 ADD24
AJ223729 CDC45L
S37730
X74794 MCM4
U63743 KNSL6
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D12614 LTA
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X51688 CCNA2
X16302 IGFBP2
X54199 GART
D00596 TYMS
X52720 PCK2
HG1322-HT1582
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U11276 KLRB1
M12125 TPM2
M95549 CDC42
J04111 JUN
M29696 IL7R
V01512
AL022710
AB011124 KIAA0552
X89277 DUSP1
AF058611 ENC1
L48215 HBB
X79536 TUBB
AF043128
D16815 NR1D2
D15050 TCF8
K00650 FOS
D87119 G33055
V01512
AJ635095 DSIPI
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AI886872 RASGRP2
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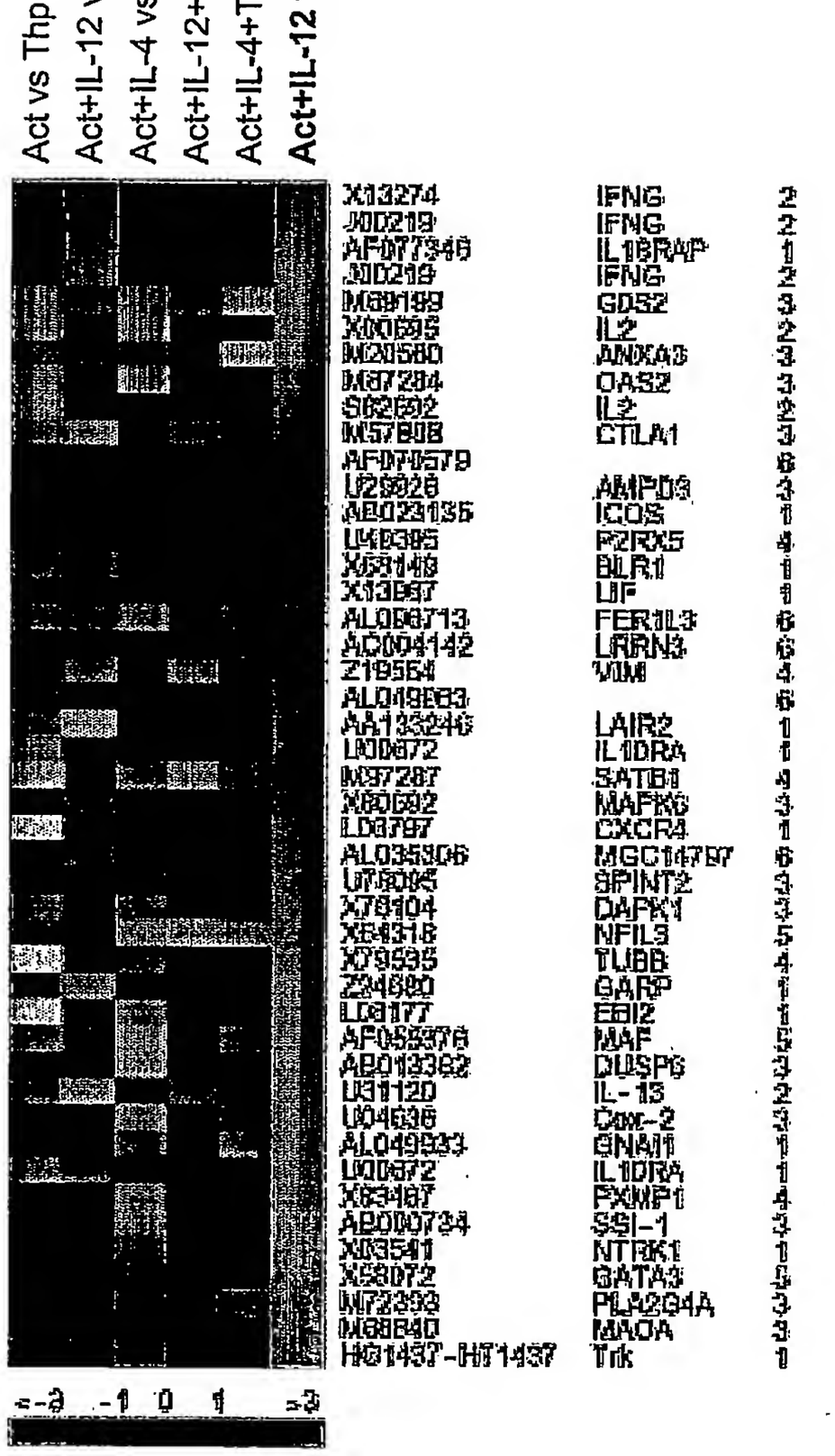
Figure 2A.


Act vs Thp
Act+IL-12 vs Act
Act+IL-4 vs Act
Act+IL-12+TGFβ vs Act+IL-12
Act+IL-4+TGFβ vs Act+IL-4
Act+IL-12 vs Act+IL-4
X13274 IFNG
J00219 IFNG
AF077346 IL18RAP
J00219 IFNG
M69199 GDS2
X00695 IL2
M20580 ANXA3
M87284 OAS2
S62692 IL2
M57906 CTLA1
AF070579
U29926 AMPD3
AB023135 ICOS
U49395 P2RX5
X53149 BLR1
X13957 UF
AL050713 FER1L3
AC004142 LRRN3
Z19554 VIM
AL049883
AA133246 LAIR2
U00672 IL1DRA
M97287 SATB1
X80692 MAFK6
L08797 CXCR4
AL035306 MGC14797
U76004 SPINT2
X78104 DAPK1
X64316 NFIL3
X79535 TUBB
Z24680 GARP
L08177 EBI2
AF055376 MAF
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U31120 IL-13
U04636 Cox-2
AL049933 GNAI1
U00672 IL1DRA
X83467 PXMP1
AB000734 SSI-1
X03541 NTRK1
X58072 GATA3
M72393 PLA2G4A
M63840 MAOA
HG1437-HT1437 Trk
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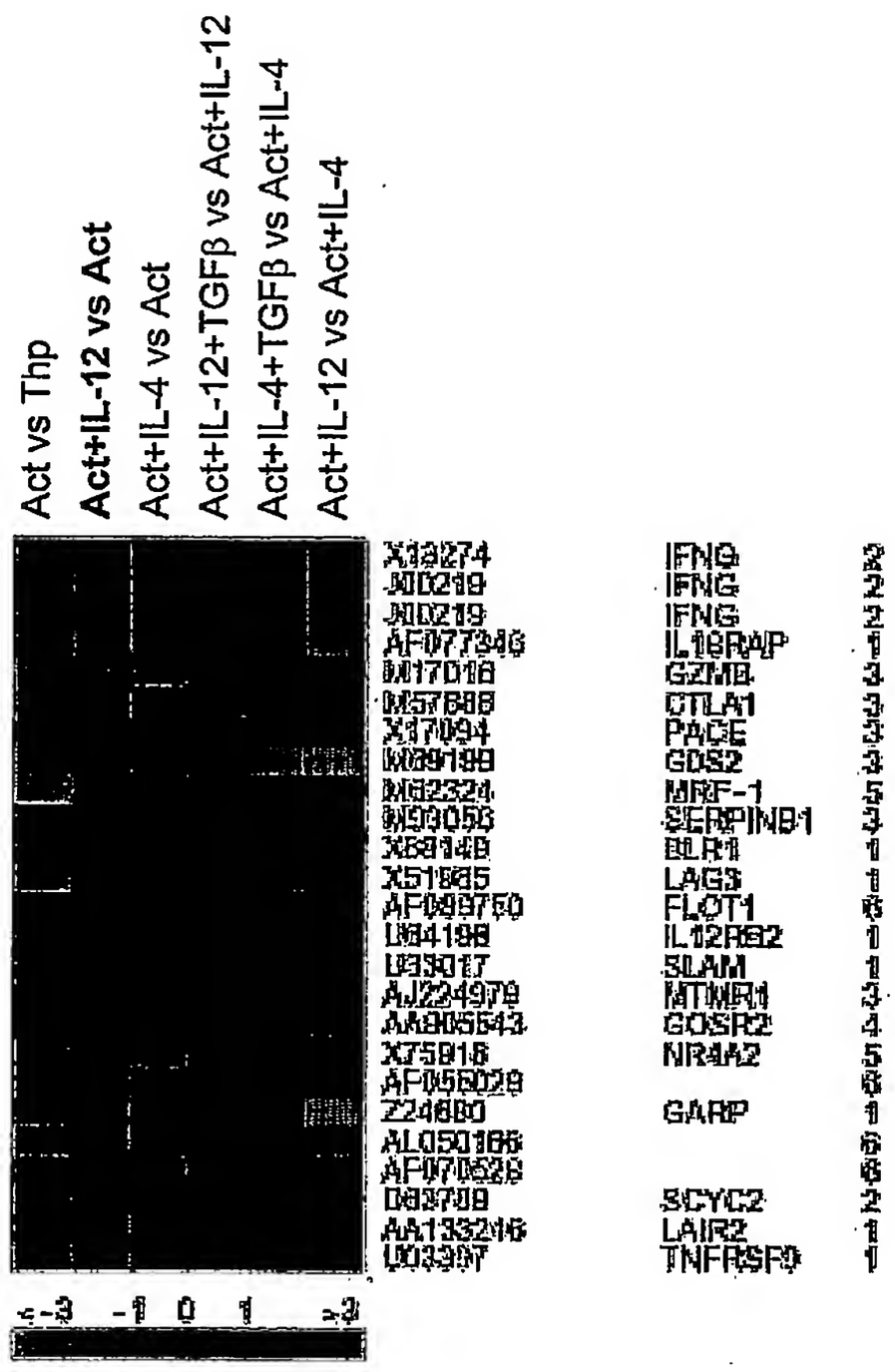
Figure 2B.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Act+IL-4 vs Act
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Act+IL-4+TGFβ vs Act+IL-4
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J00219 IFNG
J00219 IFNG
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M57688 CTLA1
X17094 PACE
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M62324 MRF-1
M93056 SERPINB1
X68149 BLR1
X51985 LAG3
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U83917 SLAM
AJ224979 MTMR1
AA905543 GOSR2
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AF056029
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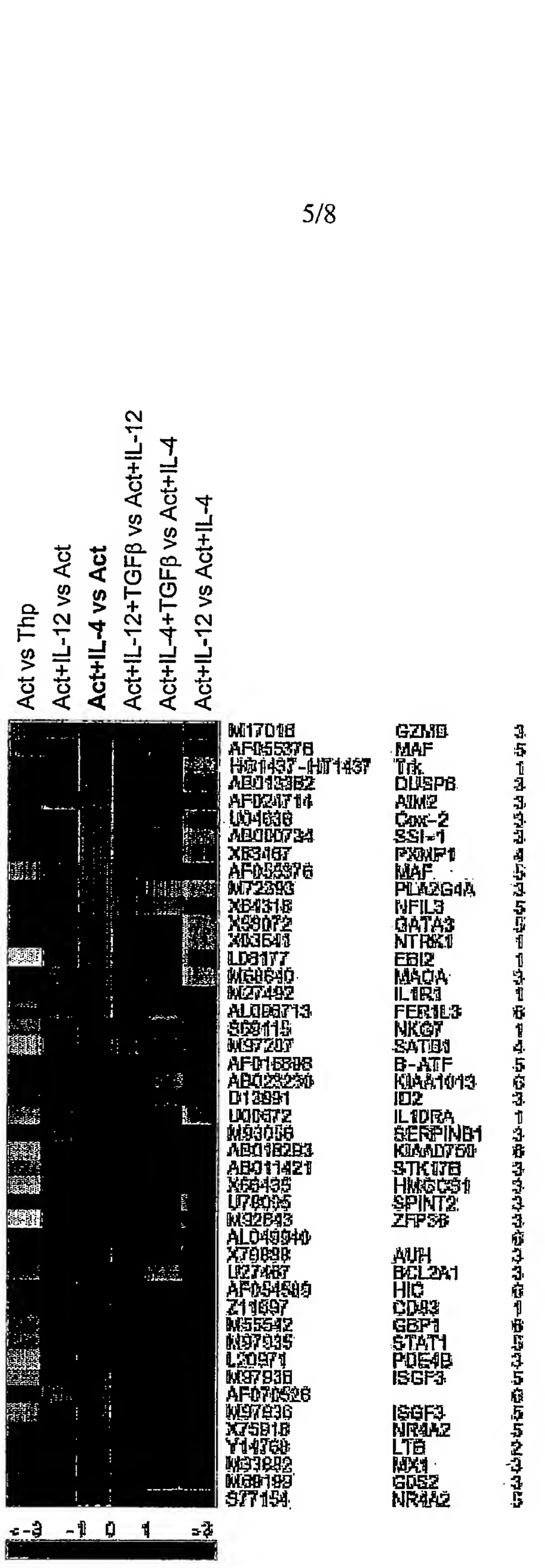
Figure 2C.


Act vs Thp
Act+IL-12 vs Act
Act+IL-4 vs Act
Act+IL-12+TGFβ vs Act+IL-12
Act+IL-4+TGFβ vs Act+IL-4
Act+IL-12 vs Act+IL-4
M17016 GZMB
AF055376 MAF
H01437-H01437 Trk
AB013382 DUSP6
AF024714 AIM2
U04636 Cox-2
AB000734 SSI-1
X83467 PXMP1
AF055376 MAF
M72393 PLA2G4A
X64316 NFIL3
X98072 GATA3
X03541 NTRK1
L08177 EBI2
M68840 MAOA
M27492 IL1R1
AL008713 FER1L3
S69115 NKG7
M97207 SAT1
AF015308 B-ATF
AB023230 KIAA1013
D13891 ID2
U00672 IL1RA
M93056 SERPINB1
AB018283 KIAA0750
AB011421 STK17B
X66435 HMGCS1
U78095 SPINT2
M92843 ZFP36
AL049940
X79888 AUH
U27467 BCL2A1
AF054589 HIC
Z11697 CD83
M55542 GBP1
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L20971 PDE4B
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AF070526
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S77154 NR4A2
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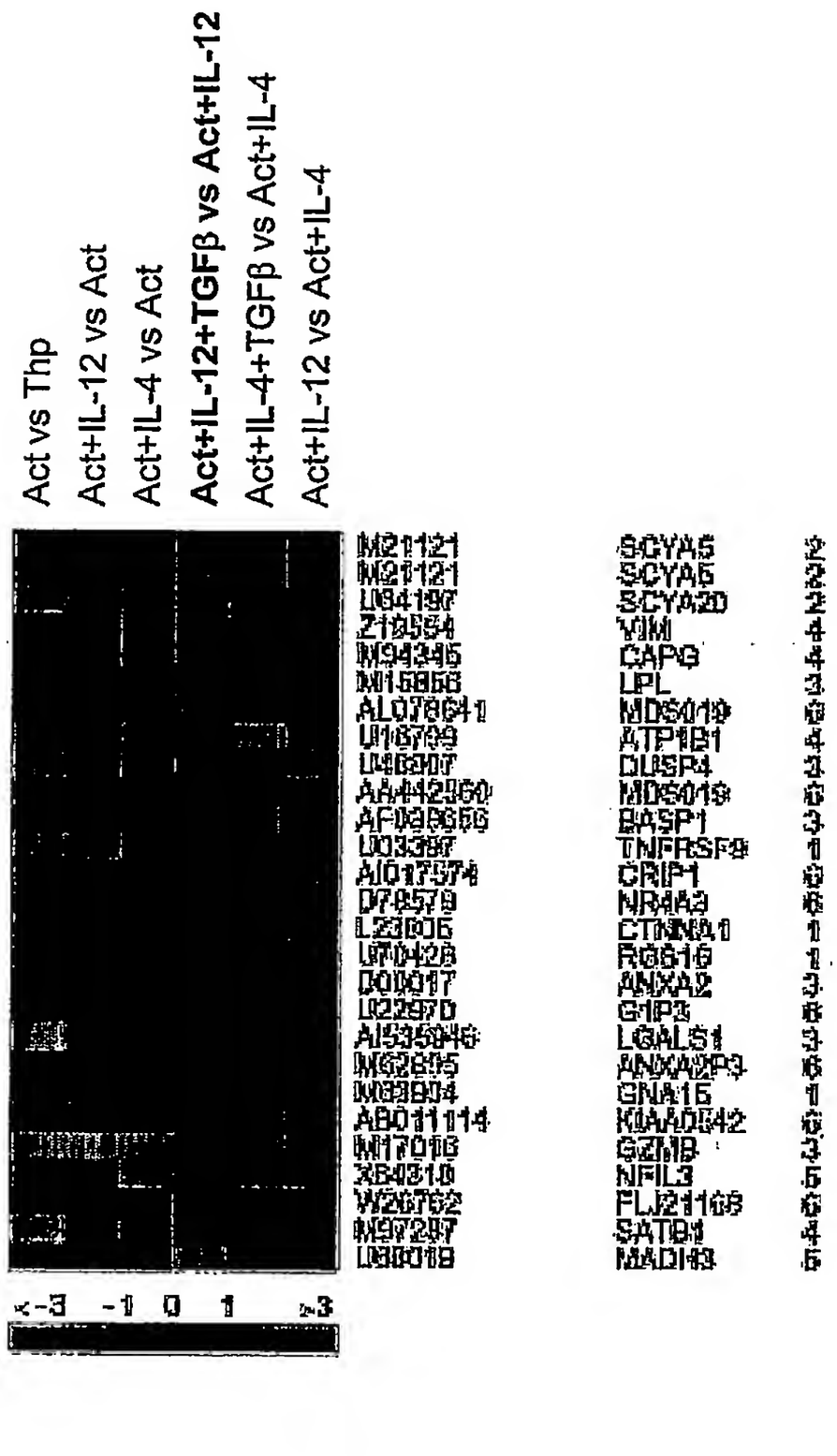
Figure 2D.


Act vs Thp
Act+IL-12 vs Act
Act+IL-4 vs Act
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Act+IL-12 vs Act+IL-4
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Z19554 VIM
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M15856 LPL
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U48807 DUSP4
AA442360 MDS019
AF098656 BASP1
U03397 TNFRSF9
AI017574 CRIP1
D78579 NR4A3
L23806 CTNNA1
U70426 RGS16
D00017 ANXA2
U22970 G1P3
AI535046 LGALS1
M62805 ANXA2P3
M33804 GNA16
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M17016 GZMB
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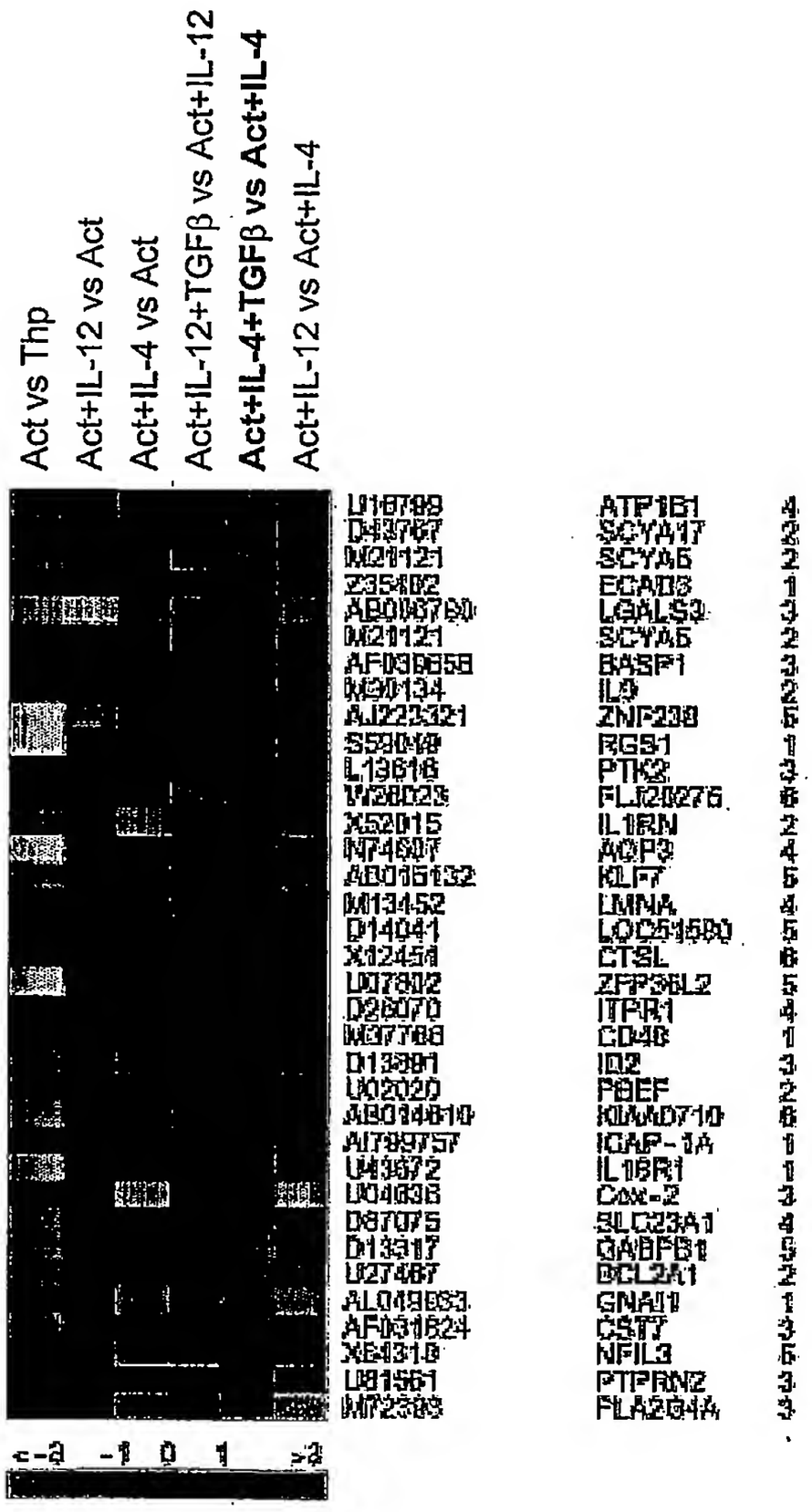
M97287 SATB1
U88018 MADH3

Figure 2E.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Act+IL-12 vs Act
Act+IL-4 vs Act
Act+IL-12+TGFβ vs Act
Act+IL-4+TGFβ vs Act
Act+IL-12 vs Act+IL-4
U18788 ATP1B1
D43767 SCYA17
M21121 SCYA5
Z35402 ECAD3
AB018790 LGALS3
M21121 SCYA5
AF038858 BASF1
M20134 IL9
AJ223321 ZNF238
S53049 RGS1
L13616 PTK2
W28023 FLJ20275
X52015 IL1RN
N74607 AQP3
AB015132 KLF7
M13452 LMNA
D14041 LOC51580
X12451 CTSL
U07802 ZFP36L2
D26070 ITPR1
M37788 CD48
D13891 ID2
U02020 PBEF
AB014810 KIAA0710
AI769757 ICAP-1A
U43672 IL18R1
U04636 Cox-2
D87075 SLC23A1
D13317 GABPB1
U27467 BCL2A1
AL049883 GNAI1
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Figure 2F.

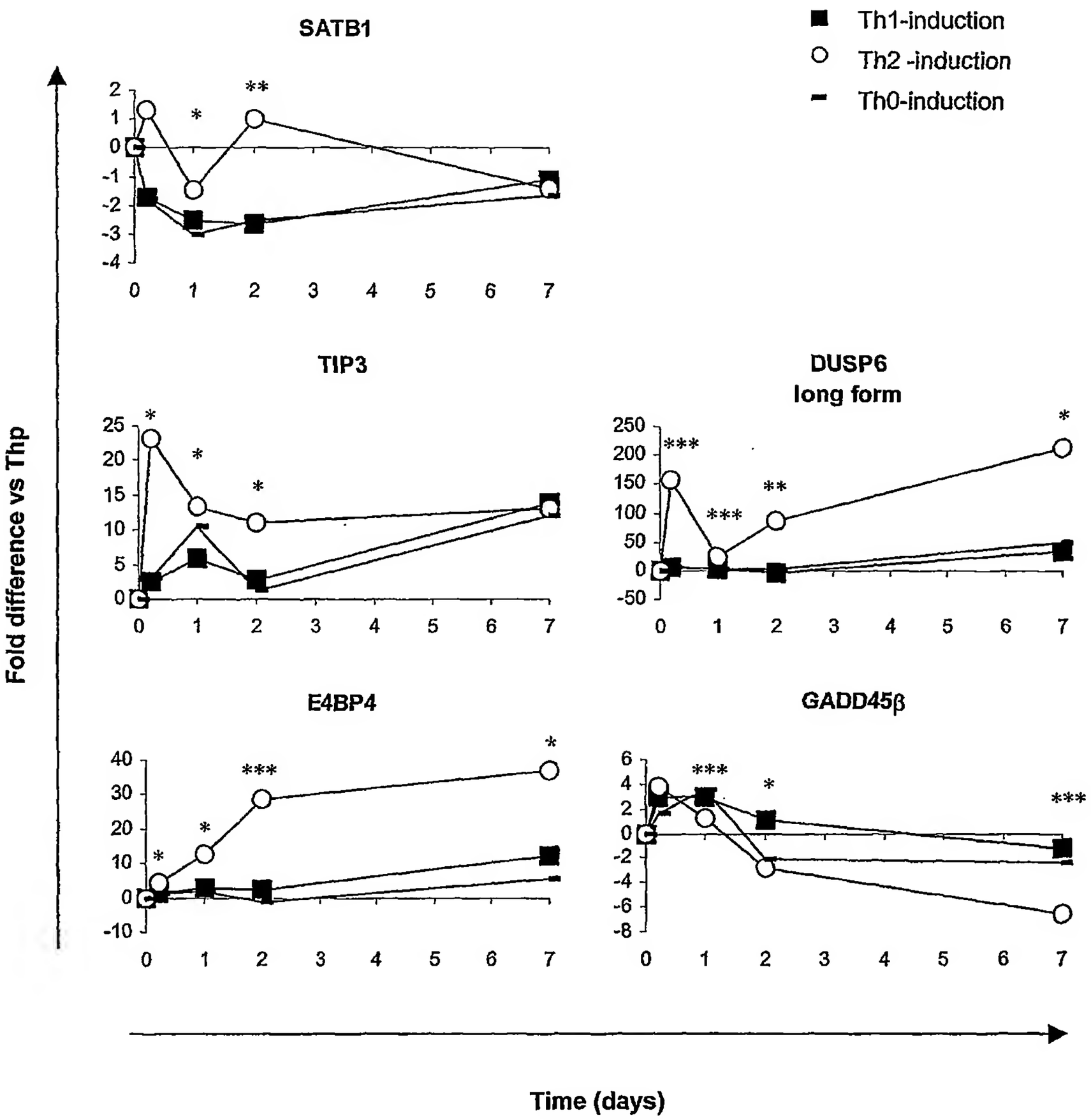
SATB1
Th1-induction
Th2 -induction
Th0-induction
TIP3
DUSP6
long form
E4BP4
GADD45β
Fold difference vs Thp
Time (days)

Figure 3.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 2004/000155

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12Q 1/68
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12Q, A61P, G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL, WPI-DATA, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Salvi H. et al: Gene expression analysis of 1,25(OH)2D3-dependent differentiation of HL-60 cells: a cDNA array study, British journal of haematology, 2002, 118, 1065-1070: See abstract. --	5-6
X	WO 0228999 A2 (GENE LOGIC, INC.), 11 April 2002 (11.04.2002), See page 1, lines 12-26, page 2, line 31 - page 3, line 8, page 4, line 20 - page 7, line 2 and Table 7, SEQ ID NO: 208 --	5-6,27

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 August 2004	07 -09- 2004
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer SARA NILSSON/EÖ Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 2004/000155

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Bellucci R. et al: Identification of target antigens associated with graft-vs-myeloma response after allogeneic bone marrow transplantation and donor lymphocyte infusion, Blood, vol. 98, nr. 11 part 1, November 2001, p. 405a. See entire abstract. --	5-6
A	WO 0188199 A2 (GENETICS INSTITUTE, INC.), 22 November 2001 (22.11.2001), See abstract, page 4, line 10 - page 8, line 20, page 10, lines 20-36, page 11, lines 25-37, page 55, lines 15-24, pages 78-80, examples E-F, and the claims --	1-4,12-31
A	US 6414117 B1 (LEVINSON), 2 June 2002 (02.06.2002), See abstract, column 1, lines 16-49, column 3, line 63 - column 4, line 1, column 4, lines 12-34 and column 76, lines 49-62 --	1-4,12-31
A	Hamalainen H et al: Distinct gene expression profiles of human type 1 and type 2 T helper cells, Genome Biol. 2001;2(7): See abstract, p. 5 right col. last paragraph and p. 9 left col. paragraph 2. --	1-4,12-31
A	WO 9957130 A1 (GENE LOGIC, INC.), 11 November 1999 (11.11.1999), See page 32, example 1, page 39, example 5 and pages 42-43, examples 8-9 --	1-4,12-31
A	Chen Z et al: Identification of IL-4 inducible genes in T lymphocytes, Journal of interferon and cytokine research, vol. 22 supplement 1, 2002. page S-172: See the entire abstract. -- --------	1-4,12-31

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI2004/000155

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 7-9
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 7-9 relate to methods of treatment of the human or animal body by surgery or by therapy or diagnostic methods practiced on the human or animal body (PCT Rule 39.1(iv)).

2. ☒ Claims Nos.: 10-11 and 7-9
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see next sheet

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see next sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-31 all partially

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

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Box No. IV Text of the abstract (Continuation of item 5 of the first sheet)

<Box II.2>

Present claims 10-11 and 7-9 relate to a product defined by reference to a desirable characteristic or property, namely that the composition "alters the expression or activity of at least one gene listed in Table 2 or Table 6". The claims cover all compounds having this characteristic or property, whereas the application lacks support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for such products. No examples of compounds are given in the description. In claim 11 it is stated that the compound is an antibody, but no specific examples of such antibodies are provided in the description. Additionally, previously known compounds may be included in the scope of the present claims. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search for claims 7-11 impossible.

<Box III>

1: Claims: 1-31 (all partially) directed to a method of identifying a compound capable of modulating the polarization of CD4+ lymphocytes comprising comparing the expression of KIAA0053 in lymphocytes contacted with the compound and induced to polarize, methods of identifying a compound that modulates the expression or activity of KIAA0053, methods for classifying lymphocytes or diagnosing immune related disorders by measuring expression of KIAA0053 and further methods comprising the step of determining the expression level of KIAA0053

2: Claims: 1-31 (all partially) directed to a method of identifying a compound capable of modulating the polarization of CD4+ lymphocytes comprising comparing the expression of LRRN3 in lymphocytes contacted with the compound and induced to polarize, methods of identifying a compound that modulates the expression or activity of LRRN3, methods for classifying lymphocytes or diagnosing immune related disorders by measuring expression of LRRN3 and further methods
comprising the step of determining the expression level of LRRN3

3-11: Claims: 1-31 (all partially) directed to a method of identifying a compound capable of modulating the polarization of CD4+ lymphocytes comprising comparing the expression of each of the other nine genes mentioned in claims 14-15 in lymphocytes contacted with the compound and induced to polarize, methods of identifying a compound that modulates the expression or activity of each of the other nine genes mentioned in claims 14-15, methods for classifying lymphocytes or diagnosing immune related disorders by measuring expression of each of the other nine genes mentioned in claims 14-15 and further methods comprising the step of determining the expression level of each of the other nine genes mentioned in claims 14-15

.../...

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI2004/000155

The searching authority could not find any same or corresponding technical feature in terms of a common property or activity, and a shared structural element essential to such a common property or activity, among the genes listed in the application. No grouping of the genes has therefore been made, and each gene is so far considered to constitute one invention (see also the motivation below).

The present application has been considered to contain 11 inventions which are not linked such that they form a single general inventive concept, as required by Rules 13.1, 13.2 and 13.3 PCT for the following reasons:

The prior art has been identified as: WO0188199 (D1).

The marker genes disclosed in D1 are identified by analyzing the changes in gene expression in response to Th1-inducing or Th2-inducing conditions. Naïve CD4+ cells are activated by contact with CD3/CD28 and either IL-12 (Th1-inducing conditions) or IL-4 (Th2-inducing conditions). RNA was isolated from samples taken at different times and RNA probes were used to probe the Affymetrix human 6800 DNA microarray set. Genes with significant differences between expression in Th1 and/or Th2 cells as compared to naïve undifferentiated CD4+ T cells are shown. Naïve CD4+ T cells are cultured in the presence of anti-CD3 and anti-CD28.
D1 discloses a method for assessing whether Th1 or Th2 cells are present in a subject comprising comparing the expression level of a marker gene in a sample from a subject and in a control sample, methods for monitoring the differentiation of naïve T cells into Th1 or Th2 cells, methods for assessing the efficacy of a test compound or therapy for modulating differentiation of Th1 or Th2 cells in a subject, methods of selecting a composition for modulating differentiation, growth or maturation of Th1 or Th2 cells and methods for assessing the potential of a test compound to trigger the differentiation of Th1 or Th2 cells from naïve T cells, all methods involving measuring the expression of genes differentially expressed in Th1 and/or Th2 cells in a sample and a control. The marker genes can be used to diagnosing conditions associated to Th-cells (e.g. asthma) by measuring the activity or expression of the marker gene in a sample. D1 also provides methods for identifying compounds which have a modulatory effect on the activity of the marker gene. See abstract, p. 4 line 10- p. 8 line 20, p. 10 line 20-36, p. 11 lines 25-37, p. 55 lines 15-24 p. 78-80, examples E-F, and the claims.

Thus, D1 shows genes being differentially expressed in CD4+ cells polarized under conditions corresponding to conditions used in the present application for identifying genes included in Tables 1-3 and 6.
The genes mentioned in claims 14-15 are differentially expressed in Th1-induced vs. Th2-induced cells (see Table 1), genes with such characteristics are shown in D1. Genes with an increased expression in Th2 cells as compared to Th1 cells are shown in D1.

.../...

Genes differentially expressed within and/or Th-cell subpopulations are also disclosed in US6414117 (D2). Murine as well as human genes are identified. The genes identified can be used diagnostically or as targets for therapeutic intervention relating to immune disorders. The genes are identified essentially as in D1. D2 provides methods for the identification of compounds which modulate the expression of genes or the activity of gene products involved in TH cell subpopulation-related disorders. See the abstract, col. 1 lines 16-49, col. 3 line 63-col. 4 line 1, col. 4 lines 12-34 and col. 76 lines 49-62.
Hamalainen H et al Genome Biol. 2001;2(7): (D3) also shows genes differentially expressed by polarized Th1 and Th2 cells. The genes are identified by polarization conditions as the ones used in the present application. See abstract, p. 5 right col. last paragraph and p. 9 left col. paragraph 2.

Invention I:

The special technical features which make a contribution over this prior art (Rule 13.2 PCT) are the following: the gene KIAA0053 is identified as being differentially expressed in CD4+ cells polarized under certain conditions.
From these special technical features the objective problem to be solved by the first invention can be construed as: providing the gene marker KIAA0053, which is differentially expressed in CD4+ cells polarized under certain conditions.

Invention II-XI:
The special technical features of claims 1-31 (all partially) are the following: each of the other nine marker genes mentioned in claims 14-15 is identified as being differentially expressed in CD4+ cells polarized under certain conditions.
From these special technical features the objective problem to be solved by the first invention can be construed as: providing each of the other nine marker genes mentioned in claims 14-15, which is differentially expressed in CD4+ cells polarized under certain conditions.

The above analysis shows that the special technical features of invention I (claim(s) 1-31 all partially) are neither the same as nor corresponding to those of inventions II-XI.

Consequently, neither the objective problem underlying the subjects of the claimed inventions, nor their solutions defined by the special technical features allow for a relationship to be established between the said inventions, which involves a single general inventive concept.

In conclusion, therefore, the XI groups of claims are not linked by common or corresponding special technical features and define different inventions not linked by a single general inventive concept.
The application, hence does not meet the requirements of unity of invention as defined in Rule 13.1 and 13.2 PCT.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/FI 2004/000155

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